Monograph on Dengue/
Dengue Haemorrhagic Fever

World Health Organization
Regional Office for South-East Asia
New Delhi
Monograph on Dengue/Dengue Haemorrhagic Fever
WHO regrets the delay in publishing this monograph.
Monograph on Dengue/Dengue Haemorrhagic Fever

Compiled by Prasert Thongcharoen, MD

World Health Organization
Regional Office for South-East Asia
New Delhi
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Preface

DENGUE virus infections are significant causes of morbidity and mortality in many areas of the world, including South-East Asia and Central and South America. The dengue virus is believed to cause two forms of clinical syndrome, namely classical dengue fever and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).

Dengue fever is a self-limiting disease and represents the majority of cases of dengue infection. In some situations it manifests life-threatening syndromes, such as the haemorrhagic and shock syndrome, which is generally known as DHF/DSS.

Besides epidemiological studies, pathogenetic mechanisms of DHF/DSS have been one of the most important issues in dengue research. Studies in South-East Asia have revealed that DHF/DSS is much more commonly observed among patients with secondary infections than among patients with primary infections. This has led to the postulation that DHF/DSS is caused by immunopathological mechanisms. Indeed, all four types of dengue virus have been found to be associated with DHF/DSS. A prevalence of Aedes aegypti and Aedes albopictus together with the circulation of dengue viruses of more than one type in any particular area tends to be associated with outbreaks of DHF/DSS. Some investigators attribute DHF/DSS to the virulence of dengue virus itself. Therefore, the pathogenesis of DHF/DSS remains unclear.

Clinical and pathological studies have shown that the major changes during infection involve the vascular system, as manifested by an increase in the permeability of the general vasculature leading to hypovolaemia and haemorrhage. Thrombocytopenia and abnormal coagulation are involved in haemorrhagic diathesis. All of these accumulated facts lead to the need for proper management and better care. Although the case-fatality rate has been reduced from almost ten per cent to less than one per cent, preventive measures through vector control and vaccine development are presently the major concerns.

In the past 30 years, research in epidemiology, vector bionomics and control, dengue virology, clinical manifestation, immunopathogenesis and vaccine development has been well documented.
This monograph is the result of the efforts of a number of contributors involved in various aspects of the study of dengue infections. Despite the delay in printing, the information in this monograph will hopefully help investigators update their current research interests.

Appreciation is attributed not only to the contributors, but also to Dr U Ko Ko, the South-East Asia Regional Director of the World Health Organization, and to Dr C.K. Sanyakorn, Dr N.K. Shah, Dr S. Pattanayak and Dr S. Jatanasen of the same office, who have made this monograph available.

Prasert Thongcharoen, M.D.
Foreword

DENGUE Fever is one of the most rapidly expanding diseases of the tropics, with over two billion people at risk of infection and millions of cases occurring every year.

The severe form of the disease, dengue haemorrhagic fever (DHF), is a leading cause of hospitalization and death among children in the South-East Asia, Western Pacific and Americas Regions. In view of the gigantic public health problem created by DHF, the interest of policy makers, scientists and administrators in the prevention and control of the disease has increased many-fold.

This publication contains information on Vector Control and Ecology, Dengue Viruses, Pathophysiology, Pathology and Pathogenesis of DHF, Management of Dengue, Clinical Manifestations and Epidemiology of DHF, and Development of DHF Vaccines. Eminent experts and renowned scientists have shared their experiences for the mutual benefit of all concerned in the control of DHF.

It is hoped that the descriptive and statistical information contained in this publication will, along with practical advice for sustained action at policy and programme levels, be of some assistance in understanding and meeting the challenges presented by the prevention and control of DHF.

Dr U Ko Ko
Regional Director
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Chapter 1
Epidemiology of Dengue and Dengue Haemorrhagic Fever

1. Dengue Haemorrhagic Fever and Dengue Shock Syndrome
   - Introduction, Historical and Epidemiological Background

by
Prasert Thongcharoen, M.D
Sujarti Jatanasen, M.D.

1.1 Introduction
The exact date when dengue fever was first recognized in the world is still obscure. Descriptions found in the early literature include, perhaps, an epidemic of “knee fever” in Cairo and its suburbs in 1779 described by Al Jabarti(1), an epidemic in Asia occurring in the same year in Batavia (Djakarta) described by David Bylon(2) and an epidemic in Philadelphia, USA in 1780 described by Benjamin Rush(3). The following is a translation of Al Jabarti’s words describing the disease.

“Year 1193 Hegira in the middle of the month of Radjab, an epidemic called ‘Knee Disease’ appeared in Cairo and its suburbs. It attacked nearly all the inhabitants, even infants. It was a sort of fever which was severe for an average duration of three days, or more or less according to personal temperament. It caused pains in joints, in the knees and swelling; and its after-effects remained over a month. It commenced suddenly and the body became hot; the head ached and the knees. Recovery was quite gained by transpiration and baths. It was an extraordinary event”.

Since then, several outbreaks have been reported from all five continents. Geographical distribution of dengue fever is world-wide, involving nearly all tropical and subtropical countries, and it has many names, for example dandy fever, denguero, denga, dunga, break bone fever, bouguet, seven day fever, bonon, chapenonada, Knieueble, Tok-kive-ana, Mal de genoux, homa mguu, and coup-d-barre.(4,5) Some unusual complications, especially haemorrhagic manifestations, have been described during epidemics of predominantly classical dengue fever; for example, during the outbreaks in Philadelphia in 1780(3), in North Queensland in 1897(6), in Hawaii in 1903(7), and in Greece in 1927-1928(8).

Classical dengue fever, as known for centuries, is characterized by fever, headache, joint and muscular pain in various parts of the body, skin rash and leucopenia. Generally, in young patients,
dengue viral infection is usually mild or unrecognizable. However, during the last three decades many types of dengue syndrome have been frequently described. The mildest form is characterized by infection of the pharyngeal wall, mild rhinitis, cough and mild gastro-intestinal symptoms, and is diagnosed clinically as pharyngitis, influenza, influenza-like disease, or even upper respiratory tract infection. The more severe forms are described as dengue haemorrhagic fever (DHF) and haemorrhagic fever with shock syndrome (DSS). Dengue haemorrhagic fever can be defined as an acute febrile illness caused by one of four serotypes of dengue virus characterized by a haemorrhagic diathesis and a tendency to develop shock syndrome that might be fatal. Thrombocytopenia, with concurrent haemoconcentration, is a constant finding. The clinical entities caused by dengue viruses are summarized in Table 1.

### Table 1. Clinical syndromes caused by dengue viruses

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Findings</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Undifferentiated Respiratory Disease</td>
<td>Fever, coryza, pharyngeal inflammation with or without cough</td>
<td>Good</td>
</tr>
<tr>
<td>Undifferentiated Fever</td>
<td>Fever with or without symptoms of multiple systems involvement</td>
<td>Good</td>
</tr>
<tr>
<td>Dengue Fever</td>
<td>Fever, myalgia and/or arthralgia and leukopenia with or without rash, headache, lymphadenopathy, biphasic fever, nausea, vomiting, positive tourniquet test, scattered petechiae and thrombocytopenia</td>
<td>Good</td>
</tr>
<tr>
<td>Haemorrhagic Fever without Shock*</td>
<td>Undifferentiated fever for two or more days, followed by two or more of the following: petechiae, purpura, ecchymosis, epistaxis, positive tourniquet test, thrombocytopenia, hepatomegaly</td>
<td>Good</td>
</tr>
<tr>
<td>Haemorrhagic Fever with Shock</td>
<td>Same as above except accompanied by shock (absence of systolic and/or diastolic blood pressure or pulse pressure 20 mm Hg or less), haemoconcentration, hypoproteinemia and/or severe gastro-intestinal bleeding</td>
<td>30 to 50 per cent mortality</td>
</tr>
</tbody>
</table>

*Chikungunya virus also causes this syndrome.

1.2 Historical Background of Dengue Haemorrhagic Fever and Dengue Shock Syndrome

Clinical records at Siriraj Hospital in Bangkok have revealed the continuous annual occurrence of typical case histories of haemorrhagic fever since 1949. From 1950 to 1957 there were more than 1500 cases of high fever with haemorrhage, with circulatory failure in some cases (Figure 1). The peak incidence each year occurred during the rainy season from July to September. Although the case fatality rate was as high as 17 per cent, preliminary investigations did not reveal any causative agent. The disease was called by Prof. Dr C. Netrasiri, a senior paediatrician, “Thai Haemorrhagic Fever” in contradistinction to the “Epidemic Korean Haemorrhagic Fever” reported from Korea during the same period of time.
The first real outbreak of haemorrhagic fever in Asia was recognized in Manila in 1954 and was reported by Quintos et al.\textsuperscript{[11]} The disease affected mainly children and was characterized by acute onset of high fever, petechial haemorrhage and shock. Quintos suggested that the disease might be transmitted by the respiratory route and named the disease “Philippine Haemorrhagic Fever”.\textsuperscript{[11]}

In the second large outbreak, two additional new types of dengue virus (types 3 and 4) were isolated from patients and\textit{Aedes aegypti} mosquitoes by Hammon and his associates.\textsuperscript{[12]}

In 1958 an outbreak of the so-called Thai haemorrhagic fever occurred in Bangkok-Thonburi and nearby areas.\textsuperscript{[13]} Almost 2500 cases and a ten per cent case fatality rate were recorded. The outbreak began in March and spread sporadically during the following three months. The number of patients increased in June and reached its peak in September. Most of the affected people were children under ten years old. Dengue types 3 and 4 were also isolated. Moreover, chikungunya virus was recovered from patients with milder symptoms.

Outbreaks of haemorrhagic fever were reported among children in Hanoi, Viet Nam, during the rainy season of 1958, and in Ho Chi Minh City (Saigon) in 1960.\textsuperscript{[9]} From the reports of the 1960 outbreak, only type 2 dengue viruses were isolated, while dengue type 1 was suspected from serological evidence.

In 1960, a small number of cases of haemorrhagic fever were observed in young adults in Singapore, and dengue types 1 and 2 were isolated.\textsuperscript{[14]} The epidemics occurred again during subsequent years, and during the epidemic seasons of 1961 and 1963 dengue viruses types 3 and 4 were isolated.\textsuperscript{[15]}

Other outbreaks of DHF/DSS have occurred in the Asian region. Dengue viruses types 1 and 4 were isolated from patients in Kampuchea in 1961.\textsuperscript{[16]} A disease clinically resembling DHF, on which no aetiological investigations were undertaken, was reported from Laos in 1962.

In Penang, West Malaysia, the disease was first recognized in 1962.\textsuperscript{[17]} Although the disease had been observed in Yangon, Myanmar, since 1963, no apparent outbreak was reported until 1970.\textsuperscript{[18]}

Other countries in the western part of the WHO South-East Asia Region, including Bangladesh, India, Sri Lanka and Maldives, were once regarded as silent areas for DHF. However, double peak epidemics of the disease were reported from Calcutta, India, between July 1963 and March 1964.\textsuperscript{[19]} Dengue virus type 2 was isolated during the first peak and chikungunya virus during the second. Severe haemorrhagic manifestations were seen during the first peak only.

In 1964, Bangladesh reported an epidemic of a disease closely related to DHF known as “Dacca Fever”, from which dengue virus type 3 was isolated.\textsuperscript{[20]}

In 1966, a small outbreak of DHF was reported from Sri Lanka. Twenty-six patients with two deaths were recorded.\textsuperscript{[21]} In the following years, only one to four cases per year were reported.

In 1968, fourteen years after the first outbreak in Manila, DHF was reported from Jakarta, Indonesia.\textsuperscript{[22]}

In Maldives, dengue-like fever occurred in Male, the capital island of the Republic of Maldives, in May.
1977 and again in May 1979. However, no outbreak of DHF was reported from Maldives until 1988 (23), when serologically confirmed DHF patients were reported from Male between the end of March and the second week of May. A total of 167 cases (with nine deaths) were admitted to Male's Central Hospital.

Dengue fever also occurs in geographical regions other than South-East Asia. Dengue occurred in epidemic form on many of the islands in the eastern part of the Pacific Ocean during 1943 and 1944. Type 1 dengue virus was isolated on one of those islands, that of Oahu, Hawaii. It is believed that this serotype was responsible for all dengue outbreaks in this geographical area at that period of time. After that, the disease disappeared and was not recognized again until the latter half of 1964 when it occurred on the island of Tahiti in French Polynesia (24). Ten years later, dengue activity had increased in the South Pacific area and had spread widely (25).

During the period 1974-1980, three epidemics of dengue fever occurred in the southern coastal area of the People's Republic of China. Over 11 per cent of the patients developed severe haemorrhage (26, 27). In 1977, a dengue pandemic began in the Caribbean. Following outbreaks on many islands, including Puerto Rico, classical dengue was introduced into South-eastern Mexico in 1978 at Tam­pico on the coast of the Gulf of Mexico (28). In September 1980, the US Center for Disease Control (CDC) reported the first case of dengue in continental USA since 1945 (29). It occurred in Brownsville, Texas. In 1981, DHF was reported from Cuba, the first outbreak of the haemorrhagic form in the American region (30).

In the African region, less information has been collected. So far, however, DHF/DSS is not a major health problem of this region (31). DHF has not been regarded as a significant problem in either the European or the Mediterranean region.

1.3 Epidemiological Background

DHF is now widespread in South-East Asia, the Western Pacific and the Caribbean. During the last three decades, more information on epidemiology has been accumulated and can be summarized as follows.

Morbidity and mortality

The morbidity and mortality rates of DHF vary widely from country to country depending on various factors, such as the immune status of the general population, the density of the vectors and the rate of dengue virus transmission, the prevalence of dengue serotypes, and meteorological conditions.

In the WHO South-East Asia Region, Thailand ranks first in the number of hospitalized DHF cases. In 1987, 174,285 cases were reported, with 1,007 deaths (2,380 DSS cases with 295 deaths), the highest record for the country (23). The morbidity rate was approximately 325 per 100,000 (130 per 100,000 in 1984; 160 per 100,000 in 1985) and the case fatality rate (CFR) was low (approximately 0.5 per cent).

Indonesia ranks second in the region according to the number of cases of DHF. Reported cases have exceeded 10,000 per annum since 1983. The highest incidence of DHF was reported in 1987, when case records of 22,765 with 1,039 deaths (CFR 4.6 per cent) were reported (23).

The average number of cases and deaths per year for the whole country of Myanmar is 2,820 and 115 respectively, ranging (respectively) from 349 and 14 (CFR 4.3 per cent) in 1973 to 729 and 222 in 1975 (CFR 3.04 per cent) (32).

In the Western Pacific Region, Viet Nam reported the first outbreak of DHF in 1958 and the number of reported cases has increased every year. The largest number of DHF cases was recorded in 1983, when there was a total number of 149,519 patients (260 per 100,000) and a case fatality rate of 1.2 per cent. The highest morbidity rate was recorded in Ho Chi Minh City (710 per 100,000 with a CFR of 0.4 per cent) (32). In 1987, an outbreak of DHF occurred with a recorded morbidity of 83,587 cases, but the case fatality rate, when compared with the three large outbreaks in 1975, 1979 and 1983, was lower. DHF ranks third among the important infectious diseases in South Viet Nam, after diarrhoea and respiratory infections. Dengue 2 (DEN-2) virus was the predominant serotype isolated during the 1987 epidemic and is widely distributed in South Viet Nam (33).
The highest number of reported cases of DHF in the Philippines was reported in 1966 at 9384 (28 per 100,000), with 250 deaths (CFR 2.6 per cent)\(^{34}\). The outbreak was mainly confined to Manila and up to 1984 this peak had not been surpassed.

In the Americas, the largest number of DHF cases occurred during 1981 in Cuba, when 344,203 cases (approximately 220 per 100,000) including 159 deaths (CFR 0.05 per cent) were recorded\(^{35}\). Dengue transmission has spread further in the Americas. A total of 42,424 cases was reported in 1982 and the number of reported dengue patients continued to increase in 1987 for the fifth consecutive year. A total of 128,430 cases with three deaths was reported in 1987\(^{36}\).

During the first outbreaks of the disease in countries in South-East Asia and the Western Pacific, the case fatality rate was high, ranging from 10 to 50 per cent, but during later epidemics the case fatality rate has generally been lower and below five per cent.

**Age, sex, ethnic group and occupation**

During the early epidemic years in each country the disease mostly affected children, and 95 per cent of cases reported occurred in the age group below 15 years\(^{13,37,38}\). However, during subsequent outbreaks in most countries, the number of case reports in older age groups has increased. The higher risk groups include children between five and nine years old. In the Philippines and Malaysia during recent years, marked increases in the number of cases in over 15 year olds have been seen, although in Thailand, Myanmar, Indonesia and Viet Nam, the majority of DHF cases are still in the age group of under 14 years\(^{22,37,39,40}\). More DHF cases have occurred in persons over the age of 15 years in the Americas than in Asia\(^{34}\).

No significant difference has been observed in sex predilection of the disease. In some countries, a slightly higher number of females with DSS has caused a higher case fatality rate in females than in males\(^{13}\).

A difference in the attack rates among different ethnic groups was noted in Singapore and Malaysia\(^{41,42}\). The disease affected Chinese people more than others. This was also observed during the early epidemics in Thailand\(^{13}\).

Occupation might also affect the incidence of DHF in some geographical areas\(^{43}\). This may be due to the fact that people in outdoor professions, such as those of policeman or gardener, are prone to exposure to *Aedes albopictus*, which is highly prevalent in some areas. In areas where *Aedes aegypti* is more prevalent, the disease occurs mostly in children and adult females who stay indoors during the daytime.

Better nutritional status seems to increase the risk of dengue shock syndrome. The disease has rarely occurred in malnourished children\(^{44}\).

**Seasonal distribution**

After the first severe outbreaks of Dengue Fever in countries in Asia between 1954 and 1958, epidemics (large or small) have occurred every year and all four dengue serotypes have been isolated. Serological evidence from the Philippines and Myanmar and hospital records from Thailand suggest that acute haemorrhagic fever occurred in sporadic form long before the first major outbreak appeared\(^{10,45-47}\). In general, epidemics have occurred every year during the rainy seasons, with some changing patterns in the later epidemics. The highly endemic areas with epidemic seasons occurring in the rainy months almost every year include the Philippines, Thailand, Myanmar, Malaysia, Singapore, Indonesia and Viet Nam, where the number of patients is correlated with the amount of rainfall. Outbreaks usually start in May and reach their peaks in July and August, before declining in October. However, in a recent epidemic in Thailand, the disease began in January\(^{39}\). In Indonesia, unlike other countries, the epidemic begins after September and reaches its peak in December, which correlates with the North-West monsoon season\(^{48}\). On the contrary, in other countries epidemics are correlated with South-West monsoon seasons.
During the early years of the outbreaks, large epidemics occurred in alternate years with small outbreaks in the intervening years. This pattern of occurrence was observed in many countries, for example, in Thailand between 1958 and 1969 and in Myanmar between 1970 and 1975. Outbreaks occurring after that period, however, have changed their patterns and become more irregular.

The vector

After the successful isolation of dengue viruses during the first epidemics of the disease in the Philippines and Thailand as mentioned above, *Aedes aegypti* was incriminated as the main vector of DHF. *Aedes albopictus* subsequently proved to be the vector transmitting DHF in Malaysia and Myanmar, where *A. aegypti* is not prevalent. In some urban areas in Singapore and Viet Nam both species are prevalent. The feeding habits of *A. aegypti* are such that it bites indoors, while *A. albopictus* bites outdoors, and is commonly found in the forest fringe, agricultural areas, and garden or plantation areas around houses. On the contrary, *A. aegypti* is more common in crowded and poor areas in the centres of cities. The incidence of DHF cases correlates well with the prevalence and density of the mosquito vectors. Age, sex and occupation distribution of DHF patients in some areas depends variably on the prevalence of these two mosquito species.

Temperature also plays an important role in the transmission of dengue virus by mosquitoes. Mosquitoes kept at 26°C fail to transmit dengue 2 virus. Hence, the low incidence of DHF in certain seasons could be explained by this observation.

Isolation of dengue virus from *Culex pipiens quinquefasciatus* and *Culex fatigans* collected from patients' houses was reported in Viet Nam and China. The virus can be isolated from mosquitoes newly-fed on patients' blood.

In both urban and rural areas, attempts were made by Rudnick to isolate dengue virus from numerous species of mosquitoes and arthropods from several habitats. However, the virus was only successfully recovered from *A. aegypti* and *A. albopictus*.

Etiology of dengue haemorrhagic fever

Although dengue types 3 and 4 were last recovered from DHF patients in Manila and Bangkok respectively in 1956, all four serotypes of dengue virus are variably isolated from DHF patients. The results of serological studies revealed that all four virus types existed before the first major outbreaks. Each of the four serotypes produces a similar disease in man. Analysis of virus isolates in Thailand has shown fluctuations of dengue virus serotype from year to year. In other countries, the prevalence of dengue serotypes varies with the area and episode. Furthermore, variation in the annual transmission rate also occurs.

Infection with any dengue serotype conveys approximately two to three months of cross protection against heterologous serotypes. Infection caused by two different serotypes appears to offer protection against DHF/DSS when reinfection with a third or a fourth serotype occurs. The immune complex mechanism between host and virus does not satisfactorily explain the pathogenesis of the disease. Epidemiological studies, supported by the WHO South-East Asia Regional Office, have been conducted in Indonesia, Sri Lanka and Thailand since 1980, and in Myanmar since 1982. The results of a study carried out in Rayong Province, Thailand,
suggested that sequential infection with dengue type 2 virus precipitated DSS\(^6\). However, dengue type 3 is believed to be responsible for DSS in Indonesia\(^4\).

**Animal reservoir**

Of several wild and domestic vertebrate species examined, only monkeys appear to be involved in the dengue cycle. Examination of monkey species in forest habitats revealed a high prevalence and significant levels of dengue antibody similar to those found in man. Dengue is a zoonosis and is maintained by a forest cycle involving wild monkeys and jungle mosquitoes. Such a cycle was demonstrated in Thailand and appears to be similar to that of jungle yellow fever in Africa, except that different local species of monkeys and mosquitoes are involved\(^3\).

**Surveillance of dengue haemorrhagic fever/ dengue shock syndrome**

The surveillance of DHF in the countries of the South-East Asia Region is mostly based on reported clinical cases. Myanmar, Indonesia, Sri Lanka and Thailand have fairly complete records, from the first recognition of the disease in their countries, regarding the number of cases and deaths in different localities. Among these reported clinical cases, less than ten per cent of patients' sera were tested by serological methods and of these about 40-60 per cent were confirmed to have dengue virus infection\(^5\). A simple and rapid method for the diagnosis of dengue virus infection is greatly needed for epidemiological surveillance of this disease.

**References**


2. Dengue and Dengue Haemorrhagic Fever in the Americas

by

D.J. Gubler, Sc.D.

2.1 Historical Background

Dengue was first described clinically in the Americas in 1780, when a large epidemic occurred in Philadelphia, Pennsylvania, USA.(1) Little information is available on the occurrence of dengue-like illnesses in the region between 1780 and the late 1820s. From 1826-1828, major epidemics of dengue-like illness occurred in the southern United States and in Caribbean Basin countries. It was during these epidemics that the name dengue came into general use to describe the disease.(2) Epidemics have subsequently occurred in the region at irregular intervals (Table 1). In recent years, however, the intervals have become progressively shorter, with major epidemics occurring every one to five years.

Table 1. History of epidemic dengue-like illness in the Americas*

<table>
<thead>
<tr>
<th>Year</th>
<th>Area Involved</th>
<th>Virus serotype</th>
<th>Years between epidemics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1780</td>
<td>Philadelphia, PA, USA</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>1817-1828</td>
<td>Caribbean-Gulf-Atlantic</td>
<td>?</td>
<td>37</td>
</tr>
<tr>
<td>1890-1891</td>
<td>Caribbean-Gulf-Atlantic</td>
<td>?</td>
<td>22</td>
</tr>
<tr>
<td>1879-1880</td>
<td>Gulf-Atlantic</td>
<td>?</td>
<td>28</td>
</tr>
<tr>
<td>1897-1898</td>
<td>Caribbean-Gulf-Atlantic</td>
<td>?</td>
<td>17</td>
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<tr>
<td>1905-1907</td>
<td>Caribbean-Gulf</td>
<td>?</td>
<td>5</td>
</tr>
<tr>
<td>1922</td>
<td>Caribbean-Gulf-Atlantic</td>
<td>?</td>
<td>15</td>
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<td>1934-1935</td>
<td>Caribbean-Atlantic</td>
<td>?</td>
<td>12</td>
</tr>
<tr>
<td>1941-1945</td>
<td>Caribbean-Gulf-Atlantic</td>
<td>DEN-2</td>
<td>3</td>
</tr>
<tr>
<td>1953-1964</td>
<td>Caribbean</td>
<td>DEN-3</td>
<td>17</td>
</tr>
<tr>
<td>1963-1975</td>
<td>Caribbean, Central, South America</td>
<td>DEN-2, 3</td>
<td>4</td>
</tr>
<tr>
<td>1977-1980</td>
<td>Caribbean, Central</td>
<td>DEN-1,2,3</td>
<td>1</td>
</tr>
<tr>
<td>1981-1988</td>
<td>Caribbean, Central, South America</td>
<td>DEN-1,2,3</td>
<td>1</td>
</tr>
</tbody>
</table>

*Adapted from Ehrenkranz et al.(2), 1971.

The virus serotypes involved in early epidemics are not known. Serologic studies in Panama suggested that dengue 2 (DEN-2) was responsible for the 1941-1942 outbreak(3), but that dengue 3 (DEN-3) was also present(4). DEN-2 was the first virus to be isolated in the American region (in Trinidad, 1953)(5). DEN-3 was responsible for major epidemics in the early 1960s and DEN-2 in the late 1960s and early 1970s(2). In 1977, dengue 1 (DEN-1) was introduced into the Americas for the first time, although retrospective serologic evidence suggests that it may have been present many years earlier(3,5). Finally, Dengue 4 (DEN-4) was introduced into the Americas in 1981(7) and since that time, three serotypes, DEN-1, -2 and -4, have been transmitted simultaneously in many of the countries of the region where Aedes aegypti occurs. DEN-3 transmission has not been documented in the Americas since 1977, but the serotype has been introduced into the region by travellers from Asia on numerous occasions in the 1980s(8).

In the 1950s and 1960s, a major effort was made to eradicate the principal urban vector mosquito of dengue and yellow fever viruses, A. aegypti, from the Americas(6). Success was variable: eradication was achieved in Mexico, Panama, Costa Rica, Colombia, Ecuador, Peru, Bolivia, Paraguay, Argentina, Chile, Uruguay, Brazil, the Cayman Islands, and Bermuda, but not in other countries of the region. The failure to eradicate A. aegypti from the whole region resulted in repeated reinvasions by this mosquito into those countries that had achieved eradication. In the 1970s, support for surveillance and control programmes was reduced, and by the end of the decade, most countries of the region had been reinfested with A. aegypti. By 1988, only Bermuda, the Cayman Islands, Costa Rica, Uruguay and Chile remained free of this mosquito species (Figure 1).
Figure 1. *Aedes aegypti* distribution in the Americas. Shaded areas represent countries with confirmed infestation in 1970 (A) and 1987 (B).
The expanding distribution of *A. aegypti* in the 1970s coincided with increased movement of dengue viruses both within and into the region, primarily by airplane travellers. The result was a constant increase in the amount of dengue transmission in the American region caused by multiple virus serotypes. In the past, most epidemics were caused by one, or occasionally two, dengue serotypes, but multiple serotypes are now endemic in most countries of the region. This has resulted in increased frequency of epidemic activity (Table 1) and the emergence of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) as a major public health problem in the Americas.

The evolving disease pattern in the Americas since the late 1970s is nearly identical to that which occurred in South-East Asia 30 years ago (Table 2). Expanded distribution and increased densities of *A. aegypti* occurred during and after World War II in Asia, and after failure of the *A. aegypti* eradication programme in the Americas. In both regions this was followed by increased transmission of multiple dengue virus serotypes resulting in hyperendemicity in many countries. In every country in South-East Asia where epidemic DHF/DSS became a major public health problem, the disease first appeared sporadically for several years, ultimately culminating in a major epidemic. Most of those countries subsequently developed a continuing cycle of epidemic DHF/DSS at three-to-four-year intervals with epidemics becoming progressively larger.

### Table 2. Similarities in the sequence of events leading to epidemic dengue haemorrhagic fever in South-East Asia and the Americas

<table>
<thead>
<tr>
<th>Events</th>
<th>South-East Asia</th>
<th>Americas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased <em>A. aegypti</em> distribution and density</td>
<td>During and following WW II 1940s-1950s</td>
<td>Following failure of eradication programme 1970s-1980s</td>
</tr>
<tr>
<td>Increased dengue transmission</td>
<td>1950s-1980s</td>
<td>1970s-1980s</td>
</tr>
<tr>
<td>Increased frequency of epidemics</td>
<td>1960s-1980s</td>
<td>1970s-1980s</td>
</tr>
<tr>
<td>Multiple dengue serotypes documented</td>
<td>1950s-1980s</td>
<td>1980s</td>
</tr>
<tr>
<td>Sporadic cases of DHF documented</td>
<td>1950s-1980s</td>
<td>1980s</td>
</tr>
<tr>
<td>First epidemic of DHF</td>
<td>1954</td>
<td>1981</td>
</tr>
</tbody>
</table>

In the Americas, the first major epidemic of DHF/DSS occurred in 1981 in Cuba. Moreover, sporadic cases of laboratory confirmed severe and fatal haemorrhagic disease associated with dengue infection have been reported in Mexico, El Salvador, Nicaragua, Jamaica, the Dominican Republic, Puerto Rico, St. Lucia, Aruba, Brazil, Surinam, Colombia and the United States (imported), while other countries such as Curacao and Haiti have reported cases that were clinically compatible with DHF, but were not laboratory confirmed (Table 3). It will be noted that most of the haemorrhagic disease has occurred in recent years. Thus, with increased incidence, there has been increased DHF/DSS in most countries where dengue has become hyperendemic. Available data suggests that the dengue disease pattern evolved in the Americas in the 1980s as it did in Asia 30 years ago. It is not yet known whether DHF/DSS will become a major public health problem in the Americas as it is in Asia.

### 2.2 Recent Changes in Epidemiology

Two countries that exemplify the changes in epidemiology that have led to the current problem in the Americas illustrate what has happened in the region as a whole.

Mexico eradicated *Aedes aegypti* in the 1960s and as a consequence had no dengue transmission during the 1960s and early 1970s. *Aedes aegypti* reinvaded the country in the 1970s and the first cases of dengue were subsequently reported from the southern state of Chiapas, in 1978. By the next year, however, dengue transmission was reported in eight states, and over the next seven years, outbreaks were reported in 24 states (Table 4). Moreover, Mexico evolved from a country of low endemicity with only a single dengue virus serotype (DEN-1) in 1978 to a hyperendemic area with three serotypes (DEN-1, -2 and -4) transmitted from 1983 to the present. The first cases of haemorrhagic disease associated with dengue in Mexico occurred in 1983 and were associated with a DEN-1 epidemic. In 1984, an outbreak of DEN-4 occurred in Merida, Yucatan, during which nine cases of haemorrhagic disease (four fatal) were
confirmed as dengue. Thus, in a period of six years, Mexico evolved from having no reported dengue to having epidemics every year, three endemic virus serotypes and sporadic occurrence of haemorrhagic disease. Currently, epidemic dengue is one of Mexico's most important public health problems.

Table 4. Epidemic dengue in Mexico, 1978-1987

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of states reporting dengue</th>
<th>Number of cases</th>
<th>Dengue serotypes</th>
<th>Hemorrhagic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>1</td>
<td>30</td>
<td>DEN-1</td>
<td>NO</td>
</tr>
<tr>
<td>1979</td>
<td>8</td>
<td>6,187</td>
<td>DEN-1</td>
<td>NO</td>
</tr>
<tr>
<td>1980</td>
<td>12</td>
<td>51,406</td>
<td>DEN-1</td>
<td>NO</td>
</tr>
<tr>
<td>1981</td>
<td>16</td>
<td>17,283</td>
<td>DEN-1</td>
<td>NO</td>
</tr>
<tr>
<td>1982</td>
<td>19</td>
<td>30,433</td>
<td>DEN-1,2</td>
<td>NO</td>
</tr>
<tr>
<td>1983</td>
<td>22</td>
<td>15,324</td>
<td>DEN-1,2,4</td>
<td>YES</td>
</tr>
<tr>
<td>1984</td>
<td>23</td>
<td>27,912</td>
<td>DEN-1,2,4</td>
<td>YES</td>
</tr>
<tr>
<td>1985</td>
<td>23</td>
<td>16,182</td>
<td>DEN-1,2,4</td>
<td>YES</td>
</tr>
<tr>
<td>1986</td>
<td>24</td>
<td>21,673</td>
<td>DEN-1,2,4</td>
<td>YES</td>
</tr>
<tr>
<td>1987</td>
<td>26</td>
<td>7,986</td>
<td>DEN-1 (DEN-2, 4?)</td>
<td>YES</td>
</tr>
</tbody>
</table>

Puerto Rico represents another example of how the epidemiology of dengue has changed in recent years. Although earlier dengue epidemics must have occurred, the first one reported in the literature occurred in 1915. It was 30 years until the next epidemic in 1945, and then a further 18 years until the next in 1963 (Table 5). The fourth major epidemic in Puerto Rico occurred six years later (1969) and, since then, endemic transmission has persisted on the island. Since 1977, Puerto Rico has experienced seven dengue epidemics caused by all four virus serotypes. Earlier epidemics were caused by a single virus serotype, but since late 1985, three virus serotypes (DEN-1, 2 and 4) have been transmitted simultaneously in Puerto Rico. Coincident with this hyperendemicity has been the emergence of DHF/DSS on the island.

Table 5. History of epidemic dengue in Puerto Rico

<table>
<thead>
<tr>
<th>Year</th>
<th>Hemorrhagic disease</th>
<th>Confirmed DHF</th>
<th>Dengue serotypes confirmed</th>
<th>Years between epidemics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1915</td>
<td>YES</td>
<td>NO</td>
<td>?</td>
<td>30</td>
</tr>
<tr>
<td>1945</td>
<td>NO</td>
<td>NO</td>
<td>?</td>
<td>60</td>
</tr>
<tr>
<td>1963</td>
<td>NO</td>
<td>NO</td>
<td>DEN-3</td>
<td>14</td>
</tr>
<tr>
<td>1969</td>
<td>NO</td>
<td>NO</td>
<td>DEN-2</td>
<td>6</td>
</tr>
<tr>
<td>1975-76</td>
<td>YES</td>
<td>YES</td>
<td>DEN-2</td>
<td>6</td>
</tr>
<tr>
<td>1977</td>
<td>YES</td>
<td>NO</td>
<td>DEN-1,3*</td>
<td>6</td>
</tr>
<tr>
<td>1978</td>
<td>YES</td>
<td>NO</td>
<td>DEN-1</td>
<td>6</td>
</tr>
<tr>
<td>1981</td>
<td>NO</td>
<td>NO</td>
<td>DEN-1,4</td>
<td>3</td>
</tr>
<tr>
<td>1982</td>
<td>NO</td>
<td>NO</td>
<td>DEN-1,4</td>
<td>1</td>
</tr>
<tr>
<td>1985</td>
<td>YES</td>
<td>YES</td>
<td>DEN-1,2,4</td>
<td>3</td>
</tr>
<tr>
<td>1986</td>
<td>YES</td>
<td>YES</td>
<td>DEN-1,2,4</td>
<td>1</td>
</tr>
</tbody>
</table>

*Predominant dengue virus in years when multiple serotypes circulated.
Figure 2. American countries with confirmed dengue hemorrhagic fever/dengue shock syndrome prior to 1981 (2A) and from 1981-1987 (2B)
In 1985, a small and limited outbreak of dengue occurred in San Juan. DEN-1 was the predominant virus serotype, but DEN-2 and 4 were also isolated. Two cases of severe haemorrhagic disease were confirmed, both of them children. One DHF/DSS case that met the World Health Organization (WHO) case definition\(^{\text{13}}\) was caused by DEN-1, and the other case (initially diagnosed as idiopathic purpuric thrombocytopenia) was caused by DEN-2 virus. In 1986, many cases of severe haemorrhagic disease were confirmed as dengue. Twenty-nine of the cases met WHO criteria for DHF/DSS, including three with a fatal outcome. Most of the severe cases in which the infecting virus serotype could be determined, including two that were fatal, were caused by DEN-4. DEN-2 virus was isolated from the other fatal case.

This pattern of sporadic DHF/DSS continued in 1987 and 1988 with smaller outbreaks. The DHF/DSS rate per 1000 confirmed dengue cases increased dramatically in 1986 and remained high\(^{\text{25-30}}\) in 1987 and 1988. Most cases of severe haemorrhagic disease in Puerto Rico, including deaths, have occurred in children under the age of 15 years, but adult cases have also been documented. The data suggest that the pattern of severe haemorrhagic disease in Puerto Rico is evolving in a manner identical to that in South-East Asia. If true, the island is at high risk for a major epidemic of DHF/DSS.

Mexico and Puerto Rico are but two examples of the changing epidemiologic patterns of dengue observed in the Americas in recent years. Limited surveillance data from other countries in Central America and South America, Hispaniola and some of the Lesser Antilles islands suggest similar changes. Moreover, the distribution of epidemic dengue is expanding rapidly. Just since 1986, major epidemics have occurred in several countries or areas that had been free of dengue for over 50 years. These include the Rio de Janeiro area of Brazil (1986), Bolivia (1987-1988), Paraguay (1988) and Ecuador (1988). Additionally, in 1987 Venezuela experienced its first dengue outbreak in ten years, and after an absence of 35 years, the United States (Texas) documented indigenous transmission twice in the 1980s (in 1980 and 1986).

In summary, several factors are responsible for the increased dengue incidence and the emergence of DHF/DSS in the American region. First, there has been a near complete breakdown in mosquito control in most countries. As noted above, \(A.\ aegypti\) has reinvaded nearly every country where it previously occurred and densities are higher. Second, increased and more rapid air travel has led to increased movement of dengue viruses within the region as well as frequent introduction of viruses from Asia and Africa. Finally, ecologic conditions have been created in tropical American cities that allow coexistence of multiple dengue virus serotypes. Prospects for changing these conditions in the near future are not good. Thus, DHF/DSS has become widespread and endemic in the Americas (Figure 2), and unless effective mosquito control programmes are implemented immediately, more frequent and larger epidemics of dengue and DHF/DSS can be expected in the future.

2.3 Clinical Illness

Severe and fatal haemorrhagic disease that appeared compatible with DHF/DSS was reported in the 1780 Philadelphia epidemic\(^{\text{11}}\), but little detailed clinical information is available about subsequent epidemics. Severe haemorrhagic disease, however, was reported during epidemics on the Gulf Coast of the United States and in the Caribbean in the late 1800s and early 1900s. Clinical descriptions of illness during epidemics over the past 50 years generally depict classical dengue fever with few complications and no fatalities from a DHF-like disease. Only in recent years have cases been described that are clinically compatible with DHF/DSS as observed in South-East Asia, with thrombocytopenia, haemoconcentration and shock (Table 3, Figure 2).

Important clinical differences between the disease in Asia and in the Americas, however, may be emerging. First, while cases of DHF/DSS that are compatible with the WHO case definition have occurred, a haemorrhagic disease characterized by severe upper GI bleeding has also been common in most American epidemics. This type of DHF was described in Tahiti\(^{\text{14}}\), and was a significant cause of
death in Indonesia\(^{(15)}\). In contrast to classical DHF/DSS, it is characterized by massive gastro-intestinal (GI) bleeding before onset of shock without any evidence of haemoconcentration. Thrombocytopenia and disseminated intravascular coagulation (DIC) are common findings. This type of haemorrhagic disease probably has a different pathogenesis than classical DHF/DSS. Moreover, it is often more difficult to manage and is associated with high mortality\(^{(16)}\).

This type of severe haemorrhagic disease, as opposed to classical DHF/DSS, may be more common in the Americas than in Asia. While classical DHF/DSS occurred in Cuba during the 1981 DEN-2 epidemic, there were also many cases with upper GI haemorrhaging\(^{(11)}\). Moreover, of 13 fatal cases in children for whom detailed clinical information was available, 12 (nine per cent) had “high digestive bleeding”\(^{(17)}\). Evidence of haemoconcentration was presented for only six (46 per cent) patients, suggesting that at least some of these children may have had a haemorrhagic disease similar to that described in Indonesia\(^{(15)}\).

In the 1984 DEN-4 epidemic in Merida, Yucatan, Mexico, nine cases (four fatal) of haemorrhagic disease were confirmed serologically and/or virologically\(^{(18)}\). There were five children and four adults. One fatal case (age nine years) had a history compatible with classical DHF/DSS with evidence of haemoconcentration. But the clinical courses of all fatal cases, including the above child, were complicated by severe GI bleeding. A recent case in Puerto Rico illustrates this type of haemorrhagic disease. The patient, a 58-year-old male with a history of six days of acute dengue-like illness, began to vomit fresh blood and was admitted to hospital. He had thrombocytopenia, prolonged prothrombin and partial thromboplastin times, and elevated fibrin split products suggestive of DIC. During the first 24 hours in the hospital, he vomited copious amounts of blood and required transfusion of eight units of whole blood. He had no history of ulcers nor of any other GI problems. Dengue infection was confirmed serologically.

Another, possibly unique, clinical feature of dengue infection in the Americas involves renal function. Confirmed dengue with jaundice and haematuria was first reported in Puerto Rico in 1975 during a DEN-2 epidemic\(^{(19)}\). Subsequently, cases of haematuria associated with dengue infection have not been uncommon in Puerto Rico (San Juan Laboratories, unpublished data). In Cuba in 1981, four of 13 fatal paediatric cases (31 per cent) had haematuria\(^{(17)}\). Of the nine confirmed cases in Mexico in 1984, two (22 per cent) had haematuria. Finally, two adult fatal cases had evidence of renal failure, jaundice and haematuria during the 1985 Aruba DEN-1 epidemic\(^{(20)}\). This type of haemorrhagic disease is seldom recognized in South-East Asia, but it has been documented in the South Pacific. Thus, during the 1971 Tahiti DEN-2 epidemic, 60 per cent (6/10) of children and 20 per cent (3/15) of adults with haemorrhagic disease had gross haematuria\(^{(14)}\).

A final difference in clinical expression of dengue between the Americas and South-East Asia concerns the age of the patients. More cases occur in persons over the age of 15 years in the Americas than in Asia. In Cuba (1981), 39 per cent of 158 fatal cases were in persons over 15 years of age\(^{(11)}\). In Mexico (1984), four of the nine confirmed cases were adults, while three fatal cases in Surinam (1982) and two fatal cases in Aruba (1985) were all adults. In Puerto Rico the majority of DHF/DSS cases have been children under the age of 15 years, but there have also been many adult cases, including some fatalities (San Juan Laboratories, unpublished data). It is likely that the high frequency of adult cases in the Americas simply reflects the lower endemicity of dengue in the region where there are still many adults that are susceptible to primary, secondary and tertiary dengue infections. If the current trend of increased endemicity continues in the Americas, however, there should be a shift of cases to lower age groups. It is of interest to note that the age group with the highest risk for classical DHF/DSS in Puerto Rico was infants less than one year of age.

In summary, severe and fatal dengue infection appears to be increasing in many countries of the Region. While some of the cases meet WHO criteria for DHF/DSS, many others do not, the main difference being the frequency of severe upper GI bleeding and haematuria. The differences outlined...
above reinforce the need for physicians in the Americas to objectively define the clinical spectrum of dengue infection in each country during epidemics caused by different virus strains and serotypes.

2.4 Geographic Distribution

As noted above, endemicity of dengue viruses has increased in most of the American region in recent years. In 1981, most countries reported the circulation of only one serotype (Figure 3). By 1985, however, multiple serotypes had become endemic in much of the region. It should be noted that the lack of dengue indicated in many of the countries in Figure 3 is the result of inadequate surveillance rather than lack of transmission. In Central America, for example, serologically confirmed dengue transmission occurs annually in most of the countries, but because appropriate samples are not taken for virus isolation, the serotypes remain largely unknown. A similar situation exists in most of the Caribbean Islands, Suriname and Guyana.

DEN-1 and 4 have been the dominant epidemic serotypes since 1981, and both have become endemic in most American countries. Although DEN-2 has also become widespread with endemic transmission in the region, this serotype has not been responsible for any major outbreaks since the Cuban epidemic in 1981. Although circumstantial, the data suggest that the strain of DEN-2 virus that circulated in the American region from 1982 to 1988 was not an epidemic strain. In Puerto Rico, for example, DEN-2 was introduced from Haiti in the summer of 1984, and DEN-1 and 4 reappeared in the summer of 1985 after three years of low-level, silent transmission. DEN-1 had been responsible for two major epidemics (in 1978 and 1981) and DEN-4 had caused the most recent outbreak in 1982. Because DEN-2 had not been associated with an epidemic in Puerto Rico since 1976-1977, therefore, it was expected that this serotype was the most likely virus to cause the next epidemic. It was DEN-1, however, that caused a small outbreak in 1985, and DEN-4 was the predominant serotype during the 1986 epidemic. DEN-2 became the dominant virus isolated only in 1987, and caused outbreaks in both 1987 and 1988, but both were relatively small. Similarly, in Mexico, DEN-2 has not caused a major epidemic even though transmission of this serotype has been documented in that country since 1982 (San Juan Laboratories, unpublished data). In contrast, repeated epidemics of DEN-1 and 4 have occurred throughout Mexico since 1978.

In 1986, a large epidemic of DEN-1 occurred in Rio de Janeiro, Brazil, an area where dengue transmission had not been reported since 1922. Subsequent outbreaks of this serotype were also documented in the states of Alagoas, Ceara, Minas Gerais and Bahia. Although only DEN-1 virus was isolated in these areas, limited serologic evidence suggested that sporadic transmission of both DEN-2 and DEN-4 may have also occurred (San Juan Laboratories, unpublished data). Why only DEN-1 caused this virgin soil epidemic is not known, but it is possible that the DEN-2 and 4 viruses did not
have epidemic potential (23). DEN-1 and 2 viruses can both exist undetected for many months in a flavivirus virgin population with silent or only sporadic transmission (26).

### Table 5. Topotype relationships of dengue 2 viruses in the Americas *

<table>
<thead>
<tr>
<th>Puerto Rico topotype</th>
<th>Jamaica topotype</th>
<th>Year of isolation</th>
<th>Virus Origin</th>
<th>Year of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puerto Rico</td>
<td>Jamaica</td>
<td>1969</td>
<td>Jamaica</td>
<td>1984</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Jamaica</td>
<td>1969</td>
<td>Jamaica</td>
<td>1984</td>
</tr>
<tr>
<td>Colombia</td>
<td>Haiti</td>
<td>1972</td>
<td>Honda</td>
<td>1984</td>
</tr>
<tr>
<td>Trinidad</td>
<td>Puerto Rico</td>
<td>1976</td>
<td>Puerto Rico</td>
<td>1984</td>
</tr>
<tr>
<td>Guatemala</td>
<td>1970</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>1983</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honduras</td>
<td>1984</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from Dr D.T. Trent.

Genetic differences between strains of DEN-2 virus from different time periods and geographic regions have been studied using the oligonucleotide fingerprint mapping technique (25,30). Two distinct topotypes of DEN-2 have been documented in the Americas (26). One, designated “Puerto Rico”, includes viruses isolated from all of the 1969 epidemics, as well as DEN-2 viruses isolated in the 1970s regardless of the country of origin (Table 6). More recent isolates from Mexico (1983) and Honduras (1984) also belong to the “Puerto Rico” topotype. DEN-2 viruses isolated from Jamaica in 1981 during the Cuban epidemic were genetically different and were designated the “Jamaica” topotype. Unfortunately, strains of DEN-2 from Cuba are not available for study, but it is likely that the Jamaica virus represents the Cuban strain because there was frequent travel between the two countries in 1981. Other DEN-2 viruses isolated from Jamaica in 1983 and from Haiti and Puerto Rico in 1984 also belong to the “Jamaica” topotype. It should be noted that none of these strains caused epidemics. Although the significance is not known at this time, it was found that the 1983 Mexico DEN-2 virus was genetically distinct from other Puerto Rico topotype viruses by antigen signature analysis (27). How, or whether, these genetic differences relate to epidemic transmission and to disease expression is not known and requires further study. Current lack of virologic identification in many epidemics and in many countries will delay these studies and, therefore, our understanding of basic epidemiologic differences that are observed during epidemics separated temporally and spatially.

### 2.5 Seasonal Distribution

Dengue transmission is seasonal in most American countries (Table 7). Epidemics in countries north of the equator have generally started during the summer months (June-August), a period that usually corresponds with the warm rainy season. In Puerto Rico, for example, epidemics in 1977, 1978, 1981, 1982, 1986, 1987 and 1988 all began in June, July or August and peaked during September-October. In Mexico and Central America, a similar seasonal transmission pattern has been observed, with most outbreaks occurring from August to November. In countries near or below the equator, transmission generally begins earlier in the year. For example, in Brazil, Bolivia, Paraguay, Ecuador and Aruba, the dengue 1 epidemics began early in the year and peaked in April-May. In most of the region, increased dengue transmission is closely linked to the rainy season and periods of weather.

*Table 7. Seasonal occurrence of representative epidemics of dengue in the American Region*

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Period of peak transmission</th>
<th>Dengue serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puerto Rico</td>
<td>1977</td>
<td>August-October</td>
<td>DEN-2,3</td>
</tr>
<tr>
<td></td>
<td>1978</td>
<td>August-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td></td>
<td>1981</td>
<td>September-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>July-October</td>
<td>DEN-4</td>
</tr>
<tr>
<td></td>
<td>1983</td>
<td>July-October</td>
<td>DEN-1,2,4</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>July-December</td>
<td>DEN-1,2,4</td>
</tr>
<tr>
<td>Cuba</td>
<td>1977</td>
<td>June-November</td>
<td>DEN-1</td>
</tr>
<tr>
<td></td>
<td>1981</td>
<td>June-September</td>
<td>DEN-2</td>
</tr>
<tr>
<td>Jamaica</td>
<td>1980</td>
<td>August-October</td>
<td>DEN-2</td>
</tr>
<tr>
<td></td>
<td>1981</td>
<td>August-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Mexico</td>
<td>1980</td>
<td>August-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>August-October</td>
<td>DEN-1,2</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>August-October</td>
<td>DEN-1,2,4</td>
</tr>
<tr>
<td>Colombia</td>
<td>1975</td>
<td>August-September</td>
<td>DEN-3</td>
</tr>
<tr>
<td></td>
<td>1976</td>
<td>September-October</td>
<td>DEN-3</td>
</tr>
<tr>
<td></td>
<td>1978</td>
<td>February-March</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Surinam</td>
<td>1982</td>
<td>January-February</td>
<td>DEN-4,4</td>
</tr>
<tr>
<td>Brazil</td>
<td>1982</td>
<td>March-October</td>
<td>DEN-1,4</td>
</tr>
<tr>
<td></td>
<td>1986</td>
<td>January-February</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Trinidad</td>
<td>1983</td>
<td>January-February</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Aruba</td>
<td>1985</td>
<td>January-March</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Bolivia</td>
<td>1986</td>
<td>February-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Paraguay</td>
<td>1987</td>
<td>February-October</td>
<td>DEN-1</td>
</tr>
<tr>
<td>Ecuador</td>
<td>1988</td>
<td>February-October</td>
<td>DEN-1</td>
</tr>
</tbody>
</table>
2.6 Mosquito Vectors

The principal mosquito vector of epidemic dengue in the Americas is A. (Stegomyia) aegypti. Originally introduced by sailing ships, this species was eradicated from many countries of the region between 1905 and the 1960s. Economic and political problems in many countries, however, resulted in decreased surveillance and control, and ultimately in reinfestation. In 1987, the geographic distribution of A. aegypti was nearly as great as it was before eradication began (Figure 1). Moreover, the increasing use of disposable, nonbiodegradable plastic containers for many consumer products has provided more abundant larval habitats, the result being increased mosquito densities.

In 1987, A. aegypti was widely distributed in the American region (Figure 1 and Table 8). All areas showing dengue transmission (Figure 3) have indigenous A. aegypti populations. In addition, areas such as the Southern United States and parts of some Central and South American countries such as Panama, Argentina and Peru have this species, but no reported dengue transmission. In the past few years, A. aegypti has reinvaded Panama, Ecuador, Peru, Bolivia, Paraguay and Argentina, leaving only the Cayman Islands, Bermuda, Costa Rica, Uruguay and Chile free of this mosquito. Cuba began an eradication programme after the 1981 epidemic, and although densities are very low, eradication has not yet been achieved.

Table 8. Mosquito vectors of dengue viruses in the Americas, 1986

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic distribution</th>
<th>Ecology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes (Stegomyia) aegypti</td>
<td>Southern United States, Mexico, Central America, Northern South America, Brazil, Bolivia, Paraguay, Ecuador, Peru, Antilles Islands</td>
<td>Urban, domestic</td>
</tr>
<tr>
<td>Aedes (Stegomyia) albopictus</td>
<td>Southern and Central United States, Brazil</td>
<td>Urban, suburban peridomestic</td>
</tr>
<tr>
<td>Aedes (Gymnomyia) meditealeae</td>
<td>Cuba, Jamaica, Hispaniola, Puerto Rico, Antilles Islands, Venezuela</td>
<td>Urban, suburban peridomestic</td>
</tr>
</tbody>
</table>

In 1985, A. (Stegomyia) albopictus was discovered in Texas and subsequently was documented in many of the Gulf Coast states as well as in Missouri, Illinois, Indiana and Ohio (Table 8). This species is also a vector of epidemic dengue and in the laboratory is a much more efficient host for dengue viruses than is A. aegypti. Thus, it is highly susceptible to oral infection, and efficiently transmits dengue viruses from human to human and transovarially. Because of this efficient host status, A. Albopictus may play an important role as a maintenance vector of endemic strains of dengue viruses.

The strain of A. albopictus introduced into the United States is cold adapted; diapause is stimulated by the shortening day length and the eggs are resistant to freezing. Thus, the species may become established in north-eastern states where LaCrosse virus is endemic. Since A. albopictus is also susceptible to infection with, and is capable of transovarial transmission of, LaCrosse virus, it may provide a link in the LaCrosse virus maintenance cycle that will put the virus in closer and more frequent contact with humans.

Evidence suggests that A. albopictus was introduced into the United States in used automobile tyres. The recent discovery of the species in Brazil and the large trade in used automobile tyres between Asia and many countries in the Americas suggest that it is only a matter of time before other countries become infested. The widespread distribution of A. albopictus in the Americas could change the ecology of dengue in the region, and provide the mosquito host that is needed to develop local maintenance cycles. This in turn could facilitate the evolution of ecologic conditions that would allow multiple serotypes to coexist in many countries of the region. Thus, the Americas will become one step closer to duplicating the epidemiologic conditions in South-East Asia.

2.7 Maintenance Cycles

Historically, dengue viruses do not appear to have been endemic in many countries of the Americas. As a result, periodic epidemics were followed by long periods without apparent transmission. It was presumed that epidemic viruses were introduced...
from other countries of the region or from other regions such as Asia. It is now known, however, that endemic transmission was occurring in many of these countries, unrecognized by the medical community. Recent examples of this are Hispaniola and Venezuela. Neither Haiti nor the Dominican Republic reported significant dengue transmission in 1984 and 1985, but active surveillance documented circulation of multiple virus serotypes in both countries (San Juan Laboratories, unpublished data). In Puerto Rico, no dengue was reported in those same years, but transmission of multiple serotypes was documented. In Brazil, the 1986 epidemic in Rio de Janeiro was caused by DEN-1, but sero-epidemiologic studies suggested that transmission of DEN-2 and 4 also occurred (San Juan Laboratories, unpublished data). In Puerto Rico, persistent low level dengue transmission was documented during the interepidemic periods of 1979-1981 and 1983-1985 only by active surveillance. It is clear from such data that dengue viruses have probably been maintained in many countries of the region in an urban cycle involving sporadic transmission to humans by A. aegypti, perhaps aided by transovarial transmission, as documented in Trinidad. On islands such as Puerto Rico, where uninterrupted endemic dengue virus transmission has persisted for 20 years, circumstantial evidence suggests that Aedes mediocutatus has played an important role in the maintenance cycle. This species, although overlapping with A. aegypti ecologically, is primarily a semi-rural/suburban mosquito, and it is common in those areas of Puerto Rico where dengue cases have been frequently confirmed during interepidemic periods. Other as yet unidentified mosquito species that fill a similar ecological niche may be playing a role in maintenance cycles in other countries.

A forest maintenance cycle such as those described in Asia and Africa has not been documented in the Americas. The only evidence to date that such a cycle might exist was the discovery of DEN-2 neutralizing antibody in persons from a remote village of the Rincón del Tigre region in the Amazon basin of Bolivia. Neither A. aegypti nor an adequate human population necessary for maintenance of dengue viruses exists in this area.

It is possible that a cycle involving Haemagogus or other canopy-dwelling mosquitoes, transovarial transmission, and monkeys exists in the forest of some American countries, but to date no studies have investigated this possibility.

2.8 Morbidity and Mortality

Accurate data on morbidity and mortality associated with dengue infection in the Americas are not available. Some countries have had disease reporting systems since increased dengue activity began in the 1970s, but the figures reported are probably gross underestimates of actual numbers of cases. Table 9 shows the total numbers of dengue cases reported for the region as a whole and the estimated incidence per 100,000 population from 1976 to 1987. It will be noted that there is a considerable variation in numbers of cases reported from year to year depending on epidemic activity in the region, and on whether seroepidemiologic surveys were carried out.

Table 9. Reported cases and rates of dengue and dengue haemorrhagic fever (DHF) in the Americas, 1977-1987

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases</th>
<th>Rate per 100,000a</th>
<th>Number hospitalizedb</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>502,026</td>
<td>309.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1978</td>
<td>69,536</td>
<td>25.27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1979</td>
<td>8,669</td>
<td>5.49</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1980</td>
<td>54,555</td>
<td>33.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1981</td>
<td>362,296</td>
<td>223.70</td>
<td>118,145</td>
<td>158</td>
</tr>
<tr>
<td>1982</td>
<td>20,244</td>
<td>15.67</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>1983</td>
<td>25,108</td>
<td>15.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1984</td>
<td>31,270</td>
<td>15.30</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>1985</td>
<td>69,960</td>
<td>42.96</td>
<td>176</td>
<td>9</td>
</tr>
<tr>
<td>1986</td>
<td>908,116</td>
<td>382.48</td>
<td>1,375</td>
<td>6</td>
</tr>
<tr>
<td>1987</td>
<td>639,317</td>
<td>394.62</td>
<td>695</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2,720,412</td>
<td>118,381</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

a Based on 1980 census data.
b Cases in 1981 were all from Cuba; cases in 1985, 1986 and 1987 were from Puerto Rico.
c Based on both reported cases and estimates made from seroepidemiologic studies.

Only in recent years have fatalities associated with dengue been documented. The largest number occurred during the 1981 epidemic in Cuba. The number of sporadic cases of DHF reported in all
countries, however, appears to be increasing each year, again depending upon the epidemic activity. In 1986, for example, six fatalities associated with haemorrhagic disease were confirmed in Brazil and Puerto Rico and unconfirmed fatal cases were reported during outbreaks in Mexico and Nicaragua. Collectively, the data suggest that in addition to higher incidence of dengue fever, severe and fatal haemorrhagic disease is also occurring more frequently in more countries.

2.9 Prevention and Control

As noted above, epidemiologic data suggest that epidemic DHF/DSS will probably become a frequent and predictable event in many countries of the American region in the 1990s, evolving in a fashion similar to the way it evolved in the South-East Asian region in the 1960s. If the course of events is not altered, DHF/DSS will most likely also become a leading cause of hospitalization and death among children in the Americas. Unfortunately, the options available for prevention and control of epidemic dengue are not good. Without vaccines for dengue viruses, mosquito control is the only viable option now available to health authorities.

Although the technical and scientific capability for control and even eradication of A. aegypti in the Americas is available, it is not used. Too much reliance has been placed on use of ultra low volume (ULV) application of insecticides for A. aegypti control in the past 20 years. During this same period of time, A. aegypti has reinvaded most of the region and the incidence of dengue has increased dramatically. This, along with the emergency of DHF/DSS in the Americas, underscores the lack of efficacy of using ULV applied toxicants alone for A. aegypti control. It is now clear that, to be sustainable, control programmes must be community-based and integrated, with emphasis on source reduction carried out by the people who create the problem.

To be effective for prevention of epidemic dengue, control must be continuous, with increased vigilance during periods when there is no apparent dengue activity. Unfortunately, it is during these interepidemic periods that mosquito control programmes are not supported because of economic problems, lack of interest by both medical and lay communities, and political pressure on health authorities to use limited resources to deal with other crises. Mosquito populations are thus allowed to increase and even spread to new areas, which then become permissive for epidemic dengue transmission. Given the economic, political and cultural realities of the American region, it is unlikely that the situation will change in the near future. Without eradication, therefore, a programme for effective epidemic prevention must rely on a combination of active surveillance for dengue viruses and severe disease, and an effective emergency mosquito control programme. Thus, if increased transmission can be detected early enough and effective emergency mosquito control implemented, major epidemics of dengue and DHF/DSS might be prevented.

To be able to predict epidemic dengue, a surveillance system must be active and laboratory-based. Ideally, each country at risk of epidemic transmission should have at least one laboratory with virologic and serologic capability for dengue diagnosis. An international surveillance network could then be coordinated by a regional reference laboratory or by an international agency such as the Pan American Health Organization (PAHO). In this manner, the distribution and spread of virus strains and serotypes could be monitored within the region and individual countries could implement A. aegypti control as necessary to prevent epidemic transmission. Unfortunately, such a network is not yet fully functional, but PAHO and the Centres for Disease Control (CDC) have been working together for the past three years to develop such a system in the American region.

Another obstacle to proper functioning of an early warning surveillance system is the tendency of health authorities to believe that the disease will not affect their countries. Thus, even though epidemics may be occurring in adjacent countries, serious local mosquito control efforts are seldom implemented until epidemic transmission has ac-
2.10 Summary and Conclusions

There has been a constant increase in the incidence of dengue in the Americas over the past 15 years. This has been caused by increased frequency of epidemic activity in most countries, as a result of increased numbers of virus serotypes circulating in the region. The change in disease ecology has resulted in the emergence of DHF/DSS in the region, first with a major epidemic in Cuba, followed by increased occurrence of sporadic cases of DHF/DSS in many countries. The sequence of events in the Americas in the 1980s was nearly identical to the pattern observed in South-East Asia in the 1950s. Prospects for prevention of epidemic DHF/DSS in the American region, therefore, are not good. In the absence of $A. aegypti$ eradication, the only hope for effective prevention and control is to develop more effective active surveillance and emergency vector control programmes.

References


3. Dengue Haemorrhagic Fever in South-East Asian Countries

by

Sujarti Jatanasen
Prasert Thongcharoen

3.1 Bangladesh

Following an outbreak of DHF in Calcutta in 1963, there was an outbreak of dengue fever in Bangladesh in 1964\(^1\). Known as "Dacca fever" with symptoms closely resembling those of dengue haemorrhagic fever (DHF), the results of the investigation showed that dengue virus type 3 (DEN-3) was the aetiologic agent. Most of the reported cases were young adults in whom some signs of bleeding diathesis and CNS involvement were seen. There was one fatal case, a 25-year old male, who died of complications from encephalitis, bleeding and vascular collapse.

Before this outbreak, sporadic cases of DHF had been reported from the border areas near Myanmar. A similar situation recurred in the following year but no laboratory confirmations were made. In 1980, four cases of clinical DHF, with one death, were again recorded without any laboratory investigation.
In 1976, a World Health Organization (WHO) short-term consultant was sent to develop a surveillance system. A small scale serological survey revealed that dengue virus was present in many parts of the country.

During 1982-83, a survey was carried out among school children in Dhaka Metropolis. Of 2465 blood samples taken, 278 were found positive for dengue 1 (DEN-1) infection by the HI test(3).

From May 1983 to April 1984, ovitrap surveys (one indoors in houses and ten outdoors) were carried out in Dhaka city to detect the presence and prevalence of *Aedes aegypti* and *Aedes albopictus*. These surveys were carried out both in the old and new areas of the city. Of the 23 areas sampled, 22 gave a positive result. The *A. aegypti* index was 16.2 and the *A. albopictus* index was 5.3. These indices were definitely low. The number of mosquitoes in the traps was also exceedingly low (*A. aegypti* per trap = 4.6; *A. albopictus* per trap = 9.48). The *A. aegypti* index in indoor ovitraps was 13.84 and that for *A. albopictus* was 2.74. Comparatively higher indices were found outdoors (*A. aegypti* = 20.03; *A. albopictus* = 9.58). More ovitraps were found positive in the congested old city areas where the water supply is sporadic and irregular. In these areas the sanitation is also poor and collection of water in gutters, discarded tins, old tyres, etc., is common. The low density of probable vector mosquitoes is said to be the cause of the absence of severe forms of dengue.

During the period 1984-1986, 21 blood samples were collected from the shishu (children's) hospitals and the Sir Salimullah Medical College Hospital, and three were found positive by the HI test. It is unfortunate that regular monitoring of the *Aedes* index, the collection of statistics of clinical cases of DHF, and the taking of serological blood samples are not carried out by the medical institutions of the four major cities.

Up to 1986, the major cities of Bangladesh, namely Dhaka, Chittagong, Rajshahi and Khulna, were free of dengue haemorrhagic fever(3).

### 3.2 Myanmar

A dengue-like illness has been reported in Yangon (formerly Rangoon) since 1954. An epidemic of dengue-like disease with marked arthralgia occurred in Yangon and several parts of the country in 1963(4). Since 1965, the Central Epidemiology Unit has been documenting the number of DHF cases admitted to hospital (Table 1)(5). There is some serological evidence to suggest the presence of chikungunya virus infection. Serological studies conducted in Yangon in 1968 revealed that 79 per cent of children under 12 years of age had antibody to dengue virus type 4 (DEN-4) and lower percentages had antibody to dengue types 3, 2 and 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases</th>
<th>No. of deaths</th>
<th>CFR (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>1966</td>
<td>10</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>1967</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>1968</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1969</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>1004</td>
<td>81</td>
<td>4.8</td>
</tr>
<tr>
<td>1971</td>
<td>59</td>
<td>34</td>
<td>4.9</td>
</tr>
<tr>
<td>1972</td>
<td>1013</td>
<td>32</td>
<td>3.1</td>
</tr>
<tr>
<td>1973</td>
<td>348</td>
<td>18</td>
<td>4.7</td>
</tr>
<tr>
<td>1974</td>
<td>2477</td>
<td>159</td>
<td>6.4</td>
</tr>
<tr>
<td>1975</td>
<td>46750</td>
<td>8431</td>
<td>5.8</td>
</tr>
<tr>
<td>1976</td>
<td>35364</td>
<td>235</td>
<td>4.3</td>
</tr>
<tr>
<td>1977</td>
<td>2029</td>
<td>82</td>
<td>4.0</td>
</tr>
</tbody>
</table>

CFR = Case fatality rate.

In Yangon, sporadic cases of DHF were recorded from 1965 to 1969 and the first large outbreak occurred in 1970 (1654 cases with 81 deaths). Between 1970 and 1974 the DHF cases were restricted to Yangon city(6). The pattern of high and low incidence occurring in alternate years was clearly seen between 1970 and 1974. Thereafter, Yangon has shown a cyclical pattern of epidemic peaks every four-five years(6). Large numbers of cases within the city limits were observed in 1970, 1974, 1979 and 1984, with the highest number of cases reported in 1974. The case fatality rate (CFR) in Yangon has varied from 5.79 per cent in 1974 to 0.46 per cent in 1982.

In 1975, the disease spread to other parts of the country with the highest incidence occurring in Mandalay, where there was an epidemic level similar to that of Yangon (Mandalay Division contributed 31 per cent and Yangon Division 29
per cent). The morbidity rate for the whole country was very high in 1975 (6750 cases with 363 deaths; 20 per 100,000 population). Since 1975, about 50 per cent of the total cases have been reported from Yangon division. In 1978, case records in Mandalay decreased, but the figures were still high in Pegu Division, Irrawaddy Division and Mon State. In 1982, there was a significant increase in the number of reported cases in Rakhaing and Kachin States. At present the disease occurs in all states and divisions except Kayah and Chin. A high incidence of DHF was seen in 1975. In 1976-1977(1 2, the attack rate was highest in the 4-8 year age group and the case fatality rate was high in the 0 to 4 year age group(3). An analysis of sero-confirmed cases showed that among the age groups of 1-4, 5-9, 10-14, and 15+ years, 1.8, 41.3, 49 and 7.7 per cent respectively of cases had occurred, indicating that the age groups of 5-9 and 10-14 years are the vulnerable age-groups(1 3). There was no difference in sex distribution.

During the first outbreak in 1970, the epidemic began in June and reached peak incidence in October(9). In the dry cool months i.e., November to February, 8.5 per cent of the annual cases were reported while the corresponding figure for the dry season, i.e., March to June, was 15 per cent. During the rainy season (July to October), 76.7 per cent of cases were reported. August was the peak month(1 1).

No virus isolation studies were carried out before 1976. The results of virus isolation studies in 1976 are shown in Table 3(13). Similar results were obtained in the virus isolation studies of 1977. Dengue viruses type 2 and type 3 were most commonly isolated from both shock and non-shock cases.

### Table 2. Dengue Haemorrhagic Fever DHF Cases and deaths, Myanmar 1970-1988 (percentage of total cases in parentheses)

<table>
<thead>
<tr>
<th>State/Division</th>
<th>Cases (%)</th>
<th>Deaths (%)</th>
<th>CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangon</td>
<td>23.52 (54.5)</td>
<td>782</td>
<td>3.3</td>
</tr>
<tr>
<td>Mandalay</td>
<td>6.13 (14.2)</td>
<td>244</td>
<td>3.9</td>
</tr>
<tr>
<td>Pegu</td>
<td>3.60 (8.3)</td>
<td>197</td>
<td>5.4</td>
</tr>
<tr>
<td>Mon State</td>
<td>3.18 (7.2)</td>
<td>93</td>
<td>3.0</td>
</tr>
<tr>
<td>Irrawaddy</td>
<td>2.89 (5.3)</td>
<td>195</td>
<td>8.5</td>
</tr>
<tr>
<td>Magone</td>
<td>1.19 (2.4)</td>
<td>63</td>
<td>7.9</td>
</tr>
<tr>
<td>Sagaing</td>
<td>0.10 (0.1)</td>
<td>41</td>
<td>4.4</td>
</tr>
<tr>
<td>Shan</td>
<td>0.07 (0.1)</td>
<td>70</td>
<td>7.5</td>
</tr>
<tr>
<td>Karen</td>
<td>0.06 (1.4)</td>
<td>26</td>
<td>4.2</td>
</tr>
<tr>
<td>Tenasserim</td>
<td>0.02 (0.1)</td>
<td>20</td>
<td>4.0</td>
</tr>
<tr>
<td>Kachin</td>
<td>0.02 (0.1)</td>
<td>58</td>
<td>15.8</td>
</tr>
<tr>
<td>Sagaon</td>
<td>0.01 (0.1)</td>
<td>15</td>
<td>15.8</td>
</tr>
<tr>
<td>Chin</td>
<td>0.00 (0.0)</td>
<td>3</td>
<td>15.8</td>
</tr>
<tr>
<td>Kayah</td>
<td>0.00 (0.0)</td>
<td>0</td>
<td>15.8</td>
</tr>
</tbody>
</table>

CFR = Case fatality rate.

In 1975, which was expected to be a year of low incidence, the number of cases increased and remained high until 1976-1977(12). Between 1976 and 1978, the attack rate was highest in the 4-8 year age group and the case fatality rate was high in the 0 to 4 year age group(3). An analysis of sero-confirmed cases showed that among the age groups of 1-4, 5-9, 10-14, and 15+ years, 1.8, 41.3, 49 and 7.7 per cent respectively of cases had occurred, indicating that the age groups of 5-9 and 10-14 years are the vulnerable age-groups(1 3). There was no difference in sex distribution.

During the first outbreak in 1970, the epidemic began in June and reached peak incidence in October(9). In the dry cool months i.e., November to February, 8.5 per cent of the annual cases were reported while the corresponding figure for the dry season, i.e., March to June, was 15 per cent. During the rainy season (July to October), 76.7 per cent of cases were reported. August was the peak month(1 1).

No virus isolation studies were carried out before 1976. The results of virus isolation studies in 1976 are shown in Table 3(13). Similar results were obtained in the virus isolation studies of 1977. Dengue viruses type 2 and type 3 were most commonly isolated from both shock and non-shock cases.

#### Table 3. Viruses isolated from acute cases of dengue haemorrhagic fever and fever of unknown origin (FUO) 1970, at the Children's Hospital, Yangon

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Number of virus strains isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shock</td>
</tr>
<tr>
<td>DEN-1</td>
<td>2</td>
</tr>
<tr>
<td>DEN-2</td>
<td>20</td>
</tr>
<tr>
<td>DEN-3</td>
<td>11</td>
</tr>
<tr>
<td>DEN-4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
</tr>
</tbody>
</table>

*Denque 2 (DEN-2) viruses were isolated from acute sera and autopsy material (liver) in two cases.

Except for the outbreak in Lashio in the northern part of Shan State in 1975, all outbreaks occurred in areas were A. aegypti breeds(13). In 1975 it was reported that A. aegypti did not breed in places above an altitude of 2700 feet above sea level. An entomological survey carried out in June 1977 in Loikow, the capital of Kayah State, situated at 1938 feet above sea level, showed A. aegypti breeding on the outskirts of the town with A. albopictus
inside the town. Although the 1975 outbreak of DHF in Lashio was not severe, evidence was obtained that DHF could occur through transmission by A. albopictus.

3.3 India

Although the first recorded outbreak of dengue fever in India was in 1812, serological surveys were first carried out in 1954, and the latter indicated that DEN-1 and DEN-2 were widespread\(^{14}\). In 1960, DEN-4 was isolated in Vellore, in the South, without any association with haemorrhagic diathesis\(^{15}\). The types of dengue virus isolated from patients in the Vellore area between 1956 and 1968 are depicted in Table 4.

### Table 4. Dengue virus isolation from patients in Vellore, 1956 to 1988

<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</td>
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<td>65</td>
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<td>50</td>
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</table>

**NOTE:** In 1965 antibody response to dengue virus in 2 cases only.

... = Data not available.

A double peak haemorrhagic fever epidemic occurred in India for the first time in Calcutta between July 1963 and March 1964\(^{16}\). DEN-2 virus strains were isolated from patients with severe haemorrhagic manifestations during the first peak and chikungunya viruses were isolated during the second peak. No severe haemorrhagic manifestation or shock case was reported among the patients with chikungunya infections. Further outbreaks occurred in 1965, 1967 and 1968. All four serotypes of dengue virus have been isolated from various parts of India\(^{17-19}\). Outbreaks of fever of unknown aetiology suspected to be DHF have been reported annually, with the affected number ranging from 1000 to 5000 and with a case fatality rate of about 0.5 per cent.

In New Delhi, outbreaks of dengue fever were reported in 1967, 1970 and 1982\(^{19}\). DEN-2 was isolated during the 1967 outbreak and DEN-1 and DEN-3 were isolated during the 1970 epidemic. An explosive outbreak of dengue fever occurred between August and October 1982. Sera were collected from 36 patients, from which 18 strains of DEN-1 and two strains of DEN-2 were recovered. This report confirmed the endemicity of dengue virus infection in New Delhi. It was noteworthy that no haemorrhagic manifestations or fatalities were recorded.

3.4 Indonesia

The first outbreak of DHF occurred in Jakarta and Surabaya in 1968, with 58 cases and 24 deaths (case fatality rate of 41.5 per cent)\(^{20}\). The following year, DHF spread to other parts of the country and the number of reported clinical cases has since increased each year. Outbreaks of the disease have involved most major urban areas as well as some rural areas. There were 10 189 reported cases with 470 deaths in 1973, and the most severe outbreak occurred in Semarang, Central Java, with 6225 reported cases. The following year, the number of patients declined. Since 1977, the disease has prevailed in 21 of 27 provinces (162 out of 300 regencies and municipalities). Forty-four per cent of the 130 million population lives in endemic areas, but there has been no report of DHF from the eastern-most parts of the country, i.e., West Irian and East Nusantengara\(^{21,22}\). The average number of cases per year between 1968 and 1972 was 474, with a case fatality rate of 13.8 per cent. A total of 6342 cases and a case fatality rate of 4.8 per cent was reported between 1973 and 1977 while 5178 cases and a case fatality rate of 4.6 per cent was reported between 1978 and 1980\(^{23}\). In 1983, about 84 per cent of 13 875 cases were reported from Java, which is the most populated area (60 per cent of the whole population). The morbidity rate for the whole country in 1983 was approximately 8 per 100 000 population. In 1987, the highest
case record was reported (22,765 cases with 1039 deaths; CFR 4.6 per cent).

Except for the year 1974, when the peak of the outbreak occurred in July, the highest incidence of DHF has usually been found during the rainy months, October to April. In 1983, the rainy season was extremely long and DHF cases occurred until June and July.

More than 90 per cent of DHF cases have occurred in children under 15 years of age. The median age of patients reported in 1979 was four-five years, but it appeared to be shifting in later epidemics with more and more cases occurring in the six-seven year age group. There was no difference in the number of males and females affected.

In 1976, two isolates of dengue virus were recovered from patients in Meduin, and were identified as type 1 and type 3. Forty-five isolates from Bantui in 1976 were identified as type 1 (eight strains), type 3 (28 strains), and type 4 (nine strains), with the rest unidentifiable. Between 1975 and 1977, 150 strains of dengue virus were isolated from the endemic area of Jakarta. All four serotypes were present with type 3 predominant (23 strains of type 1, 38 strains of type 2, 59 strains of type 3, nine strains of type 4, with the rest unidentifiable). Aedes aegypti mosquitoes, the principal vectors, are distributed widely throughout the country.

3.5 Maldives

An outbreak of dengue-like fever occurred in Male, the capital island of the Republic of Maldives, in May 1977 and again in May 1979. The outbreak spread to Eydhafushi Island, Baa Atoll. Between 12 May and 7 June 1979 about 213 cases were recorded as dengue-like illness. In 1983, dengue-like fever was reported in March-April. In September 1983, ten cases of dengue-like fever were reported from Eydhafushi, and twenty cases from Kendhoo Island. Aedes aegypti and Aedes albopictus were widely present in Baa Atoll as well as on other Islands.

An outbreak of fever cases was reported from Male between the end of March and the second week of May 1988; 167 cases were admitted to Male Central Hospital and nine deaths occurred. Serological (HI) tests on blood specimens collected from admitted fever cases were positive for dengue infection. Eight of the ten paired sera were tested by the Medical Research Institute, Colombo, Sri Lanka. In this outbreak of dengue, severe and fatal cases were mostly amongst children. This was the first occurrence of dengue haemorrhagic fever in Male.

3.6 Sri Lanka

Sri Lanka appears to have had endemic dengue fever since the early part of this century, but no DHF was reported until 1965. Serological and virological evidence to this effect was first recorded in 1960. Chikungunya virus infection, as confirmed by serological testing, was demonstrated in 1964 but the virus was not isolated from patients until 1965.

In 1965, two deaths from DHF were reported, while thirteen cases with five deaths were seen in 1966. The number of patients increased to 29 with eight deaths in 1967; while seven cases of DHF with two deaths were reported in 1968. No case of DHF was reported from Sri Lanka between 1971 and 1976. In 1977 four cases of DHF were diagnosed, all children under 14 years of age. Secondary dengue virus infection was confirmed serologically. Two children were seriously ill with shock and bleeding, but all survived. Thereafter between one and four cases have been reported annually. For an island with a population of 15 million, this incidence of DHF is not really significant.

Serological studies have revealed the presence of dengue virus activity in all major towns located below an altitude of 4000 feet and all four types of dengue virus prevail. Between August 1982 and February 1983 an outbreak of dengue fever without haemorrhagic diathesis started in Colombo North and spread mainly to Colombo Central. In
the outbreak, which appeared to be due to DEN-2, thirteen strains of dengue virus were isolated.

Generally, the peak of the disease occurs in June and is associated with the South-West monsoon, which commences in late April and has maximum rainfall in May and June. When the disease appears later in the year it is related to the North-East monsoon, which prevails from October to December.

Even though Aedes aegypti and Aedes albopictus are prevalent in the country, Sri Lanka is still regarded as a silent area for DHF/DSS. A research programme on DHF/DSS surveillance was supported by the WHO South-East Asia Regional Office (WHO-SEAR) from 1980-1985.

### 3.7 Thailand

Before the first large outbreak of DHF/DSS in 1958, approximately 50 to 100 cases diagnosed as "influenza with haemorrhage" were included in the hospital records of Siriraj Hospital in Bangkok. After the 1958 outbreak in Bangkok and its suburbs, the disease spread to adjacent provinces in the Central region in 1961. In 1964, a major outbreak occurred in big cities in northern and north-eastern Thailand. The highest record of DHF/DSS (5403 cases with 216 deaths) was reported in 1964 from Bangkok. Since 1968, there have been reports of the disease from almost every province of the country. During the first ten-year period (1958-1967), epidemics occurred in alternate years with peaks during the rainy seasons. Even in low epidemic years, the number of patients increased yearly except in 1986. The number of cases recorded was 69 597 in 1984, 80 076 in 1985, 27 837 in 1986 and 174 285 (with 1007 deaths) in 1987, which latter was the highest figure ever reported in the WHO South-East Asian region. The case fatality rate was approximately ten per cent in 1958, but gradually decreased to below one per cent by 1980.

After 1968, the epidemic pattern of alternate years changed and became irregular for the whole country. Case records in Bangkok remained high but did not exceed the 1964 number and followed the country-wide pattern. Since 1973, the number of patients in the north-eastern part of the country has increased significantly every year, and now comprises almost 50 per cent of the cases for the whole country. In the early epidemic years, the number of cases in the dry seasons (November to March) was very low (below 100 cases per month), with most reported cases occurring in Bangkok. In other provinces the number of cases was less than ten per month during the dry-cool season (November to February).

Between December and January from 1979-1985, the reported number of DHF cases was higher than 100 per province per month in four-five provinces of the central and north-eastern regions. The total reported number of patients in the dry-cool season was, therefore, more than 500 cases per month between November and February. For the whole country, case records reached 2345 in December 1984, and 1859 in January 1985. This changing pattern is under investigation.

In Thailand, Aedes aegypti is the main vector of DHF. Isolation of dengue virus from Aedes albopictus was also reported when a small outbreak of DHF occurred in the insular setting of Koh Samui in southern Thailand.

Surveillance of DHF cases in Thailand, which has a mainly clinical basis, has been conducted for several years. For surveillance purposes, all cases with undifferentiated fever, pyrexia of unknown aetiology, or fever persisting for about five to seven days in which common cold and upper respiratory tract infection have been excluded, have to be notified. Serological examination has revealed that 40-50 per cent of these cases are dengue virus infection. In this regard, the over-reporting and under-reporting of DHF cases in Thailand seem to balance.

Virus isolation from patients admitted to Bangkok Children's Hospital has been performed almost every year at AFRIMS (formerly called SEATO Medical Research Laboratory). The analysed results are shown in Figures 2 and 3 in chapter 1.1. It should be pointed out that DEN-2 has predominated every year, while DEN-1 and DEN-4 have appeared during alternate years.
After 1981, more typical clinical symptoms than those suggested by the WHO criteria for clinical DHF were observed. At the Children's Hospital in Bangkok more frequent encephalopathy and jaundice were reported. The age group between five and nine years remained the high risk group, but in recent epidemics more children over 14 years and adults have been affected.

Chikungunya viral infection has been confirmed by virus isolation or by serologic test in clinically diagnosed DHF patients in about 0.5 to four per cent of cases. This figure shows that the incidence of chikungunya in Thailand is lower than in Myanmar.

Table 5 shows the number of DHF cases and deaths reported from Myanmar, Indonesia and Thailand between 1958 and 1987.

### Table 5. Annual dengue haemorrhagic fever cases, deaths and case fatality rates (CFR) in Myanmar, Indonesia and Thailand, 1958-1987

<table>
<thead>
<tr>
<th>Year</th>
<th>Myanmar</th>
<th>Indonesia</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>No. of deaths</td>
<td>CFR (per cent)</td>
</tr>
<tr>
<td>1958</td>
<td>1654</td>
<td>81</td>
<td>4.80</td>
</tr>
<tr>
<td>1959</td>
<td>588</td>
<td>34</td>
<td>5.06</td>
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<tr>
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<td>1913</td>
<td>32</td>
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<td>3.17</td>
</tr>
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<td>3.00</td>
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<td>1524</td>
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<tr>
<td>1971</td>
<td>2356</td>
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<td>111</td>
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</tr>
<tr>
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<tr>
<td>Total (mean)</td>
<td>50755</td>
<td>2065</td>
<td>4.08</td>
</tr>
</tbody>
</table>

References


43. Sangkwawibha P, Department of Medical Sciences, Ministry of Public Health, Thailand. Personal communication 1986.


4. Epidemiology of Dengue in the Western Pacific Region

by
S.K. Lam, T. Pang
T. Umenai

4.1 Introduction

Dengue virus is probably the most important virus transmitted to man by arthropods. Although reliable data are not available, it can be assumed that dengue fever (DF) and dengue haemorrhagic fever (DHF) are leading causes of hospitalization and death among children in Asia.

Classical dengue fever was first recognized more than two centuries ago when, in 1776, epidemics were recorded in Java and Egypt. Siler et al. believed that dengue originated in 'tropical America' as opposed to claims of Mattingly who believed that it originated in South-East Asia. However, it was a few years later that Rush gave the first accurate clinical description of true dengue when an outbreak occurred in Philadelphia in 1790. In the 19th and 20th centuries, extensive outbreaks were reported from tropical and subtropical areas on all continents and from many sub-continents and islands in the South Pacific and the Caribbean. Epidemics were reported in the Southern United States (1920, 1922), Australia (1925-1926, 1942, and 1954-1955), South Africa (1926-1927), Greece (1927-1928) and Japan (1942-1945).

In Queensland the first outbreak of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) might have occurred in 1877 but it was not recognized as such until later. DHF outbreaks were also recorded in refugee-swollen Athens and Piraeus. In Bangkok, children were hospitalized with DHF/DSS-like syndromes during every rainy season from 1950-1965. DHF/DSS cases were recognized in South-East Asia after the 'Philippines haemorrhagic fever' in 1953 and the 'Thai Haemorrhagic Fever' in 1958. In 1956 alone at least 1000 children with haemorrhagic fever were hospitalized in Manila and, from some of these cases, Hammon et al. recovered dengue 2, 3 and 4 viruses.

The increase in the incidence of dengue may be attributed to two important factors, namely, the rapid growth and urbanization of people in the tropics and the increased frequency and speed of human travel. Unlike other arthropod-borne viruses, dengue virus does not require a non-human host, and transmission from man to man by mosquitoes occurs freely in urban settings.

The early history of dengue epidemics in southern Asia followed the invasion by Aedes aegypti and its spread. Dengue infection has occurred widely throughout the Western Pacific region except in areas climatically unsuitable for the vectors.

Data concerning DF/DHF in countries of the Western Pacific vary from scanty to good, according to the country. This may be a reflection of the importance of the disease as a public health problem or of the interest in the disease as such. In this review, we have tried to collate the most recent data available to us on the epidemiology of dengue in the Western Pacific region.

4.2 Singapore

Dengue haemorrhagic fever (DHF) first appeared in Singapore in 1960 when 70 hospitalized cases were reported between June and October of that year. The disease then became endemic on the island and periodic outbreaks were recorded. Severe outbreaks occurred during 1966-68 (2312
cases, 63 deaths) and 1973 (1187 cases with 27 deaths).

Minor outbreaks occurred in 1978 (with 332 cases), in 1986 (with 354 cases) and again in 1987 (with 436 cases). The total number of cases and deaths reported in various years is shown in Table 1.

Table 1. Dengue Fever/Dengue Haemorrhagic Fever in Singapore

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases</th>
<th>Number of deaths</th>
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</thead>
<tbody>
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<td>42</td>
<td>12</td>
</tr>
<tr>
<td>1966</td>
<td>630</td>
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<td>126</td>
<td>9</td>
</tr>
<tr>
<td>1986</td>
<td>354</td>
<td>-</td>
</tr>
<tr>
<td>1987</td>
<td>436</td>
<td>-</td>
</tr>
</tbody>
</table>

*Source of data (7), (10), (11), (12), (13)
... = Data not available

For the year 1985, the overall morbidity rate was 4.9 per 100 000 population. Age specific morbidity was high in the 5-14 (7.2 per 100 000), 15-24 (6.5 per 100 000) and 25-34 (6.9 per 100 000) year age groups(7). The male to female ratio was 1.6 : 1 and the ethnic-specific morbidity rate for Chinese (4.3 per 100 000) was higher than that observed among Indians (3.0 per 100 000) and Malayans (1.8 per 100 000). Cases were reported throughout the year with a peak in October, and five foci of transmission were identified in various parts of the island, the highest number of cases being in the Taman Jurong area(7). The Aedes house index in this area was 12.3 per cent at the beginning of the outbreak but was reduced to 0.7 per cent following prompt implementation of vector control measures. During 1985, blood specimens taken from 98 of the 126 notified cases were tested for serological confirmation. Of the 48 paired sera, 35 (72.9 per cent) showed a four-fold rise in haemagglutination inhibition (HI) antibody titres. In the case of single specimens, 30 out of 50 (60 per cent) showed a titre of 1:1280(7). There is no information on the serotypes of dengue isolated.

A recent seroepidemiological survey tested 425 blood samples from the healthy population for HI antibody titres to dengue 2 (DEN-2) virus(9). Of the 425 sera tested, 231 (54.4 per cent) possessed no HI antibody to DEN-2 virus. All 99 young children (below four years of age), 23 of 24 children of four years of age (95.8 per cent) and 48 of 53 children in the five to nine year age group (90.6 per cent) were seronegative. There was then a sharp increase in prevalence of HI antibody so that by the age of 40 years all were seropositive (44 per cent, 70 per cent, 78 per cent, 86 per cent and 100 per cent in the 10-14, 15-19, 20-29, 30-39 and 40 years age groups respectively). These results confirmed the low level of dengue virus transmission in the country after more than a decade of intensive vector control measures. Children below ten years of age appeared to be at greatest risk.

Although DHF is no longer a major cause of paediatric morbidity and mortality in Singapore, the island remains both receptive and vulnerable to epidemics of DHF for several reasons e.g. waning herd immunity among the population (especially among children and adolescents), the high probability of imported cases due to the large influx of travellers to and from neighbouring countries and the persistence of Aedes mosquitoes(7).
4.3 Viet Nam

The first case of DHF was reported in the South in 1963 and in the North in 1969. DHF has since become a major public health problem with the annual number of cases being the highest in the Western Pacific region. From 1975 to 1983 epidemics occurred almost annually, and the incidence varied from 52.3 to 260.6 per 990 000 population\(^{14}\). Severe epidemics occurred in the whole country in 1975, 1977, 1978, 1979, 1980 and 1983. The most severe epidemic occurred in 1983 in all parts of Viet Nam, and 149 519 cases with 1798 deaths (case fatality rate of 1.2 per cent) were reported. In South Viet Nam, most of the DHF cases were children in the five to six year age group whereas in the North adults were also involved\(^{14}\). There appeared to be equal numbers of male and female cases.

It has also been observed that epidemics of DHF occur mainly in the rainy season, the breeding period of \textit{A. aegypti}. All four serotypes of dengue virus have been isolated from human cases of DHF as well as from \textit{A. aegypti} mosquitoes\(^{14}\). There have also been claims that dengue-1 (DEN-1) and DEN-2 viruses have been isolated from \textit{Culex fatigans}\(^{14}\). The positivity of paired sera sent for serological confirmation by the HI test ranged from 34.3 per cent to 55.4 per cent.

A summary of DHF activity in Viet Nam from 1975 to 1983 is given in Table 2.

Table 2. Dengue Haemorrhagic Fever (DHF) in Viet Nam, 1975-1983\(^{*}\)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of DHF cases</th>
<th>Number of DHF deaths</th>
<th>Cases/100 000 population</th>
<th>Case fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>30 900</td>
<td>418</td>
<td>77.0</td>
<td>1.4</td>
</tr>
<tr>
<td>1976</td>
<td>25 722</td>
<td>887</td>
<td>62.3</td>
<td>2.7</td>
</tr>
<tr>
<td>1977</td>
<td>40 544</td>
<td>572</td>
<td>80.4</td>
<td>1.4</td>
</tr>
<tr>
<td>1978</td>
<td>43 864</td>
<td>616</td>
<td>84.5</td>
<td>1.4</td>
</tr>
<tr>
<td>1979</td>
<td>63 670</td>
<td>1174</td>
<td>121.3</td>
<td>1.8</td>
</tr>
<tr>
<td>1980</td>
<td>95 146</td>
<td>817</td>
<td>177.1</td>
<td>0.85</td>
</tr>
<tr>
<td>1981</td>
<td>48 523</td>
<td>408</td>
<td>84.2</td>
<td>1.15</td>
</tr>
<tr>
<td>1982</td>
<td>36 808</td>
<td>581</td>
<td>70.9</td>
<td>0.8</td>
</tr>
<tr>
<td>1983</td>
<td>146 519</td>
<td>798</td>
<td>260.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^{*}\)Source of data: (14), (15).

4.4 Philippines

Following the initial recognition of DHF in the Philippines in 1953-54, dengue has remained highly endemic and epidemics have occurred every three to five years. The 1956 epidemic in Manila had a case fatality rate of eight per cent. The biggest DHF epidemic occurred in 1966 when 9384 DHF cases and 250 deaths were reported (case fatality rate of 2.7 per cent). Since then, although DF/DHF appears to have decreased in importance, DHF cases have continued to occur particularly in Manila. The total number of cases of DF/DHF covering the period 1956-1984 is given in Table 3.

Table 3. Dengue Haemorrhagic Fever (DHF) in the Philippines\(^{*}\)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of DHF cases</th>
<th>Number of DHF deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>1 207</td>
<td>72</td>
</tr>
<tr>
<td>1958</td>
<td>84</td>
<td>34</td>
</tr>
<tr>
<td>1960</td>
<td>551</td>
<td>42</td>
</tr>
<tr>
<td>1961</td>
<td>1 459</td>
<td>30</td>
</tr>
<tr>
<td>1964</td>
<td>756</td>
<td>186</td>
</tr>
<tr>
<td>1966</td>
<td>9 364</td>
<td>250</td>
</tr>
<tr>
<td>1968</td>
<td>1 116</td>
<td>115</td>
</tr>
<tr>
<td>1970</td>
<td>922</td>
<td>80</td>
</tr>
<tr>
<td>1972</td>
<td>1 570</td>
<td>83</td>
</tr>
<tr>
<td>1974</td>
<td>1 655</td>
<td>153</td>
</tr>
<tr>
<td>1975</td>
<td>875</td>
<td>70(^{a})</td>
</tr>
<tr>
<td>1977</td>
<td>759</td>
<td>72</td>
</tr>
<tr>
<td>1978</td>
<td>410</td>
<td>37</td>
</tr>
<tr>
<td>1979</td>
<td>508</td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>1981</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>1982</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>1983</td>
<td>1 664</td>
<td>130</td>
</tr>
<tr>
<td>1984</td>
<td>5 575</td>
<td>89</td>
</tr>
</tbody>
</table>

\(^{a}\)Source of data: (10), (15), (17).

A summary of DHF activity in Viet Nam from 1975 to 1983 is given in Table 2.

Recent data from the Philippines suggest that 1980, 1981 and 1985 saw little disease activity whilst more serologically confirmed cases were detected in 1982, 1983 and 1984 (Table 4). The age distribution of recent cases of DHF indicates that more than 40 per cent of cases are in the five to nine years age group (Table 5). The sex ratio of cases shows approximately equal distribution of cases between males and females (131 males and 141 females between 1982-84). The years 1982 and 1983 showed the typical pattern of increased...
disease activity during the rainy months. In 1984, however, most cases were seen during the dry months; the reasons for this are unknown\(^{16}\). All four serotypes of dengue have been isolated in the Philippines and, in recent years, dengue-1, 2 and 3 (DEN-1, -2 and -3) appear to have been equally prevalent (Table 6).

<table>
<thead>
<tr>
<th>Year</th>
<th>Clinical diagnosis</th>
<th>Serologically confirmed</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>152</td>
<td>95</td>
<td>66.45</td>
</tr>
<tr>
<td>1981</td>
<td>68</td>
<td>20</td>
<td>29.4</td>
</tr>
<tr>
<td>1982</td>
<td>250</td>
<td>122</td>
<td>48.8</td>
</tr>
<tr>
<td>1983</td>
<td>244</td>
<td>118</td>
<td>48.4</td>
</tr>
<tr>
<td>1984</td>
<td>26</td>
<td>9</td>
<td>28.6</td>
</tr>
</tbody>
</table>

*Source of data: (16)

Table 4. Dengue infections confirmed by serology*

4.5 Pacific Islands

The past 10 to 15 years have witnessed the introduction and dissemination of all four dengue serotypes in the Pacific Islands. DEN-2 virus was introduced into the South Pacific in 1971 and DEN-1 in 1974. The introduction of DEN-1 was followed by major outbreaks of dengue in the Pacific Islands during 1974-75. The outbreak started in the Marshall Islands in early 1974, was in Nauru by mid-1974 and later that year in Kiribati and Tuvalu Islands. By January 1975 the epidemic had spread to Vanuatu and Fiji and by mid-1975 to Tonga, French Polynesia and both Western and American Samoa (Table 7). Dengue-4 (DEN-4) virus was introduced into Tahiti in 1979 and caused a major outbreak in the South Pacific. The island of Niue, which has a population of 3000, reported 616 cases of DF/DHF with four deaths. Other

<table>
<thead>
<tr>
<th>Year</th>
<th>1982</th>
<th>1983</th>
<th>1984</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 4</td>
<td>7</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>5-9</td>
<td>28</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td>10-14</td>
<td>23</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td>Over 15</td>
<td>7</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

*Source of data: (18)

Table 5. Age distribution of serologically confirmed dengue cases: 1982-1984*

<table>
<thead>
<tr>
<th>Country/Area</th>
<th>Reported cases of DF/DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Samoa</td>
<td>230</td>
</tr>
<tr>
<td>Cook Island</td>
<td>-</td>
</tr>
<tr>
<td>Fiji</td>
<td>72 506</td>
</tr>
<tr>
<td>French Polynesia</td>
<td>1 460</td>
</tr>
<tr>
<td>Kiribati</td>
<td>2 28</td>
</tr>
<tr>
<td>Nauru</td>
<td>-</td>
</tr>
<tr>
<td>New Caledonia</td>
<td>11</td>
</tr>
<tr>
<td>New Zealand</td>
<td>-</td>
</tr>
<tr>
<td>Niue</td>
<td>-</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>9</td>
</tr>
<tr>
<td>Samoa</td>
<td>2 547</td>
</tr>
<tr>
<td>Tokelau</td>
<td>67</td>
</tr>
<tr>
<td>Tonga</td>
<td>8 354</td>
</tr>
<tr>
<td>Vanuatu</td>
<td>1 412</td>
</tr>
</tbody>
</table>

*Source of data: (12), (15), (17), (18), (21)

... = Data not available

Table 7. Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) in the Pacific Islands, 1975-1984*

Table 6. Dengue viruses isolated in the Philippines, 1983-1984*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>1983</th>
<th>1984</th>
<th>Total</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue-1</td>
<td>54</td>
<td>34</td>
<td>88</td>
<td>35.6</td>
</tr>
<tr>
<td>Dengue-2</td>
<td>56</td>
<td>12</td>
<td>70</td>
<td>22.4</td>
</tr>
<tr>
<td>Dengue-3</td>
<td>40</td>
<td>33</td>
<td>73</td>
<td>31.7</td>
</tr>
<tr>
<td>Dengue-4</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>6.7</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>81</td>
<td>249</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Source of data: (16)
South Pacific Islands were also affected (Table 7). In 1982 a DF epidemic occurred on the Solomon Islands with 1800 reported cases; DEN-3 virus was isolated and the vector responsible was found to be A. albopictus\(^1\)

### 4.6 China

Dengue fever was first reported in China in the 1940's during World War II when epidemics occurred in Central and Southern China. For unknown reasons the disease disappeared for 30 years and reappeared in 1978-1979 in the Foshan District of Guangdong Province, Southern China. In this DF/DHF outbreak caused by DEN-4 virus, a total of 22,122 cases were reported with 14 deaths\(^1\). The majority of cases observed were mild cases of dengue fever with a few presenting with mild haemorrhagic phenomena. No genuine dengue haemorrhagic fever or shock syndrome was documented\(^1\). In the spring and summer of 1980, another epidemic of dengue fever caused by DEN-3 occurred in Guangxi Province and Hainan Island, also in Southern China. Another major epidemic seems to have occurred in China in 1983 with 85,293 cases and a reported 3032 deaths\(^2\). However, little information is available on this outbreak.

During the 1978-1980 period three serotypes were responsible for disease activity: DEN-4 in 1978, DEN-1 in 1979 and DEN-3 in 1980\(^3\). DEN-4 and DEN-1 were transmitted mainly by A. albopictus and were restricted largely to coastal countries near Hong Kong. DEN-3, however, spread to a wider area including Hainan Island and the coastal regions of Guangdong and Guangxi provinces, and was mainly transmitted by A. aegypti\(^4\).

### 4.7 Malaysia

In Malaysia, A. aegypti was found in Port Swettenham in the early 1900s, but was not found inland. Dengue fever was first recognized in Malaysia in 1902\(^5\) but DHF was first reported only in 1962\(^6\). The disease was made notifiable by law in 1971. In March 1954, an outbreak of febrile illness was reported in a girl's school in Kuala Lumpur from which the first dengue virus was isolated in Malaysia and identified as DEN-1.

All the four dengue serotypes are endemic in Malaysia\(^7\). Outbreaks of dengue have been reported mainly from the more developed and populated states of peninsular Malaysia. A major outbreak occurred in 1973 with 969 cases. During this outbreak a case fatality rate of 5.6 per cent was recorded\(^8\). Most of the confirmed cases were in the younger age group i.e. 54 per cent were under ten years. During the following year, the situation worsened when 2200 cases with 104 deaths were reported. From 1973-1985 (Table 8), the number of DF/DHF cases averaged 740 a year. These reported figures are on the low side as many cases do not get reported to the Ministry of Health. The mortality rate declined slowly over a period of 12 years, from a high DHF case fatality rate of 9.53 per cent in 1975 to 3.3 per cent in 1984, and from an overall DF/DHF case fatality rate of 4.94 per cent in 1975 to 0.7 per cent in 1984. Although only 354 cases of DF/DHF were reported in 1985, there were 11 deaths among the 112 DHF cases, giving a case fatality rate of 9.8 per cent. This is a disturbing trend in the clinical manifestation of the disease in the country.

### Table 8. Incidence of reported dengue cases and deaths in Malaysia, 1973-1987

(Figures in parentheses denote deaths)

<table>
<thead>
<tr>
<th>Year</th>
<th>DF</th>
<th>DHF</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973</td>
<td>515</td>
<td>909 (54)</td>
<td>1,424 (54)</td>
</tr>
<tr>
<td>1974</td>
<td>718</td>
<td>1,452 (104)</td>
<td>2,166 (104)</td>
</tr>
<tr>
<td>1975</td>
<td>400</td>
<td>430 (41)</td>
<td>830 (41)</td>
</tr>
<tr>
<td>1976</td>
<td>349</td>
<td>441 (43)</td>
<td>790 (43)</td>
</tr>
<tr>
<td>1977</td>
<td>1,340</td>
<td>368 (38)</td>
<td>1,708 (38)</td>
</tr>
<tr>
<td>1978</td>
<td>1,025</td>
<td>454 (43)</td>
<td>1,479 (43)</td>
</tr>
<tr>
<td>1979</td>
<td>445</td>
<td>367 (26)</td>
<td>812 (26)</td>
</tr>
<tr>
<td>1980</td>
<td>359</td>
<td>300 (23)</td>
<td>659 (23)</td>
</tr>
<tr>
<td>1981</td>
<td>234</td>
<td>270 (17)</td>
<td>504 (17)</td>
</tr>
<tr>
<td>1982</td>
<td>2,160</td>
<td>860 (54)</td>
<td>3,020 (54)</td>
</tr>
<tr>
<td>1983</td>
<td>575</td>
<td>215 (10)</td>
<td>790 (10)</td>
</tr>
<tr>
<td>1984</td>
<td>547</td>
<td>150 (13)</td>
<td>697 (13)</td>
</tr>
<tr>
<td>1985</td>
<td>245</td>
<td>142 (11)</td>
<td>387 (11)</td>
</tr>
<tr>
<td>1986</td>
<td>1,059</td>
<td>310 (9)</td>
<td>1,369 (9)</td>
</tr>
<tr>
<td>1987</td>
<td>1,858</td>
<td>304 (7)</td>
<td>2,162 (7)</td>
</tr>
</tbody>
</table>

Source: Ministry of Health, Malaysia.

Since dengue became notifiable, there are data to suggest that major outbreaks of dengue occur every four years (1974, 1978, 1982, 1986). The
Dengue activity in 1986 appeared to spill over into 1987 when 2002 cases were notified with seven deaths.

A recent age stratified survey based on 5138 specimens collected from one dengue sensitive study site in Malaysia showed that children under one year of age had antibody to one or more dengue serotypes and that this declined to 8.6 per cent at one year due to loss of maternal antibody. The antibody prevalence then increased by an average of five per cent every year until the age of 18 when all subjects tested were positive. Of the 2620 males and 2416 females studied, there was no significant difference in antibody prevalence between the two sexes.

A socio-demographic survey and a survey on knowledge, attitude and practices of the study population were carried out. The data were analysed against the serological findings and the following were noted:

- Children from smaller families had lower antibody prevalence than those from large families.
- There was no difference in antibody prevalence based on income analysis.
- Twenty-eight per cent of children from families with good knowledge of the disease had antibodies to dengue compared to 51 per cent of those from families with little knowledge.
- Although piped water was available, 80 per cent of the households surveyed stored water in open containers.
- Children from homes with receptacles and containers in which mosquitoes could breed had a higher prevalence of antibody.
- Antibody prevalence was related to the A. aegypti Breteau and Premise indices.

The epidemiology of dengue in Malaysia is related to two rainy seasons, the SW monsoon in the first half of the year and the NE monsoon in the second half of the year. The endemicity is low from January to April and increases, reaching a peak in July/August. Smaller peaks may appear between October and November. This variation may be related to the storage of water during the drought season from January-April and the drizzling rainfall before the heavy monsoons, which create suitable breeding places for the vectors.

DF is most prevalent in adults, while DHF is more prevalent in children. In 1975, 64.7 per cent of dengue cases were in the under 15 years age group, but in the peak year of 1982 only 42.5 per cent of cases were in this age group. Similarly for mortality, 51.4 per cent of deaths in 1982 occurred in people over 15 years, whereas in the past it was rare for adults to die of dengue disease. The disease has been showing a milder spectrum with less mortality since 1984. The decline in mortality can be attributed to increased awareness of the disease, early medical attention and changing patterns of the disease. More deaths have occurred among the Chinese and this can be attributed to their lifestyle, customs and beliefs, which delay them from seeking proper medical attention and referral.

The two vectors of dengue in Malaysia are A. aegypti and A. albopictus. Aedes aegypti is a domiciliary mosquito and is found both inside and outside the home. It is usually associated with DHF in urban settings. Aedes albopictus, a local species, is prevalent in peri-urban areas and is associated with dengue fever.

The density of the vector mosquitoes is related to climatic conditions and the habit of storing water. Aedes aegypti prefers clean clear water with low oxygen partial pressure e.g. rainwater. The Aedes premise index and the Breteau or container index are used to monitor the density of mosquitoes for preventive actions. Periodic house inspections and entomological surveys carried out under existing law (Destruction of Disease Bearing Insect Act, 1975) have not generated useful indices. Outbreaks tend to occur in areas with high Aedes densities. However, areas with low Aedes indices (of five per cent or less) are not exempt.

4.8 Australia

Aedes aegypti is believed to have become established in northern Australian settlements in the 1860s.
Lumley and Taylor documented regular epidemics of dengue, commencing in 1873 when eight clinical cases were imported into Sydney from Mauritius. Local transmission was probably first recorded from Townsville in 1879 but the outbreaks on the Charters Towers gold fields during 1885-1886 are better remembered because of their extensiveness and the description by Hare of what is considered to be the first DHF case. By 1926, clinical dengue had been reported in four Australian states, coincident with the distribution of *A. aegypti*. Lumley and Taylor reported on the large outbreak of dengue in 1941-1944, which spread from Queensland to New South Wales due to the movement of armed services personnel. From 1954-1955, north Queensland was stricken by DEN-3 virus and some 15,000 of 40,000 residents of Townsville were infected. In ensuing years, *A. aegypti* was reduced and this led to the decline of dengue in the country.

From 1981-1983, there was an upsurge of dengue activity in Queensland. Breteau indices of over 100 were not uncommon, a level said to be associated with dengue haemorrhagic fever in Thailand. Observations related to Pathogenesis of a "new" dengue disease. *Amer J Publ Hth* 1965, 55: 1386-95.


5. Dengue in Africa

by

Michel Cornet

5.1 History

The first epidemic attributed to dengue in Africa occurred in Cairo (Egypt) in 1779, as stated by Christie(1). This author also gives an account of epidemics starting in Zanzibar, on the East coast of Africa, in 1823 and 1870. The term dengue has its origin in Zanzibar, where the disease was called "denga" during the 1870 epidemic. In Durban (Natal, South Africa) a big epidemic was recorded in 1926-1927 by Edington(2). During World War II and immediately after, several epidemics were recorded from the coast of East Africa and the neighbouring islands(3-5). In West Africa, epidemics were recorded in Upper Volta in 1925(6), in Dakar (Senegal) in 1928(7) and on ships after calling at West African harbours(8,9).

With our recent knowledge of arbovirus circulation in Africa, it is more likely that most of these epidemics were not true dengue but other arbovirus diseases which may cause urban epidemics. Carey(20) stated that the Zanzibar epidemics were due to chikungunya virus. Several flaviviruses have also been involved in epidemics during recent years (Zika, West Nile) as well as other arboviruses transmitted by anopheline mosquitoes, such as O'Nyong'Nyong, Bwamba or Tataguine.

In fact, the first formally recognized epidemic of dengue in Africa was in Durban, where a retrospective serological survey showed evidence of anti-dengue 1 antibodies in people born before the epidemic(11). It must be mentioned that this is the only record of African dengue where severe haemorrhagic syndromes have been recognized(2).

The first isolation of dengue virus (DEN) in Africa occurred in Nigeria, when a DEN-2 strain was obtained from a patient. Many other strains of DEN-1 and DEN-2 viruses were isolated from patients in Nigeria between 1966 and 1975. These results focused attention upon dengue in Africa, and in recent years the four virus types have been found in Africa. The most interesting observation concerns the discovery of an important sylvatic circulation of DEN-2 virus in at least four West African countries in 1980-81. Previously this sylvatic cycle was known to occur only in Malaysia and Viet Nam.

5.2 Dengue Review by State

East and South Africa

South Africa (Natal)

A big epidemic occurred in 1926-1927 in the town of Durban, where more than 40 000 cases were suspected. Haemorrhagic syndromes seem to have been frequent and probably also shock syndromes since Edington(2) mentioned "many serious cases of cardiac decompensation". The real nature of this epidemic was only recognized in 1956, when Kokernot et al(11) found DEN-1 antibodies in the blood of nine 26-year-olds. No other positive response for DEN-1 or DEN-2 viruses was found outside the Durban area and, considering the commercial relations of this harbour, it is most likely that the virus was introduced from South-East Asia.

More recently, a case of DEN-1 was diagnosed in Durban by virus isolation from a patient just returned from India; several other cases were suspected in the same area(12).

Indian Ocean Islands

During World War II or immediately after, epidemics occurred in the Comoro Islands(2,4) as well as in Diego-Suarez in Madagascar(5). None of these epidemics was serologically investigated and their dengue nature has remained doubtful.
In 1976-1978, the Seychelles Islands experienced an extensive epidemic which affected at least 60 per cent of the population\(^{13}\). This epidemic started in December 1976, at the beginning of the rainy season, reached its peak in February 1977 and lasted until September 1977; sporadic cases were observed in 1978 and there was a small resurgence epidemic between September 1978 and January 1979\(^{14}\). The rainfall is 2000-3000 mm per year and occurs from December to March. The epidemic spread through nearly all of the inhabited islands of the archipelago. Twenty-three DEN-2 virus isolates were obtained from the 36 tested sera and a retrospective serological survey in the town of Mahe showed only two negative responses for DEN antigen out of 231 tested sera\(^{13}\). Most of the sera were positive for the four antigenic types. Clinically, most of the cases were typical dengue, the rash being less frequent than usual. No haemorrhagic syndrome or shock syndrome was noticed, but there were deaths caused by encephalitis and meningitis of "probably viral" origin. Cases occurred in indigenous people as well as in expatriates. The most likely vector was \textit{Aedes albopictus}, which is very abundant during the rainy season; twenty-three tested pools of this species yielded five isolates; isolates were also made from two of eight \textit{Culex quinquifasciatus} pools and from one of the two \textit{Styloconops spinosifrons} (Ceratopogonidae) pools, but the virus might have resulted from recently ingested blood.

At the same time (1978) an epidemic was observed in La Reunion Island, and the nature of the epidemic was suspected after a single DEN-2 virus isolate was made at the Pasteur Institute of Madagascar from the blood of a patient who had just arrived from La Reunion. The number of cases was estimated at about 160,000, that is to say 30 to 35 per cent of the total population, and no DHF or DSS was recorded\(^{16}\).

A few cases were recorded from Mombasa during World War II\(^{4}\). The results of a serological survey carried out in 1966-1968\(^{16}\) showed that "dengue transmission has taken place in the coastal area of Kenya in the past". Antibodies for alphaviruses (chikungunya and O'Nyong'Nyong) were also present in more than half of the tested sera of two of the three areas investigated.

Between March and October 1982, DEN-2 virus isolates were obtained from seven of 43 sera from acutely ill patients\(^{17,18}\). The epidemic spread along the coastal area of Kenya and neighbouring Somalia. Clinical investigations were few and medical personnel did not notice an increase of febrile illness, but, sometimes, only an increase of cases diagnosed as "clinical malaria". No survey was done to investigate an increase of mortality during the outbreak but some doctors noticed "an increase in cases of encephalitis and/or circulatory collapse", as had already been seen in the Seychelles Islands. The epidemic was extensive since the antibody prevalence rate rose from 7.6 per cent in June to 52 per cent in October.

In Somalia, three cases were confirmed in expatriates in May 1983 by a specific rise of DEN-2 IgM, but in May 1984 no evidence of dengue transmission was found during limited surveillance\(^{19}\).

In January 1984 investigations were started in Port Sudan (Sudan), on the Red Sea coast, to determine "the cause of perennial epidemics of fever"\(^{19}\). Twenty virus strains were isolated, of which 17 were DEN-2 and one was DEN-1. No other data were given concerning the clinical and epidemiological aspects of transmission.

**Mozambique**

The Health Ministry of Mozambique recorded an epidemic in Pemba (Province of Cabo Delgado), which began in October 1984 at the beginning of the rainy season, and lasted until March 1985\(^{20}\). At least two deaths were attributed to this disease initially thought to be malaria, and DEN-3 virus was isolated and identified in the sera of four patients. A decrease in blood platelets was fre-
quently noted. This is the first record of DEN-3 virus in Africa.

West Africa

Nigeria

Nigeria was the first African country to carry out exhaustive studies on dengue. Between the first virus isolate, in 1964, and 1975(21-24), dengue viruses were recovered from 68 patients (38 DEN-1 and 30 DEN-2). Most of the cases were children less than four years old and most of the samples examined were from children; cases in adults were few and most of them were hospital or laboratory personnel(25). Diseases were mild, generally milder in young patients, and no DHF or DSS was noticed. Three strains of DEN virus were obtained from children with febrile convulsions(26). A single case with low hemorrhagic tendency was diagnosed in an American physician.

Epidemiologically, all cases diagnosed were from the rain forest area, mainly from Ibadan because surveillance was higher in this city. The virus was isolated all the year round, except in the dry months of February and March, with the peak occurring in August, which corresponded to a short dry season between the two rainy seasons. A single isolate of DEN-2 virus was obtained from Aedes aegypti caught in Ibadan in August 1969(21).

The first suspicions of a sylvatic cycle of dengue were aroused after serological surveys were carried out to investigate yellow fever on the Benue Plateau(27) and in the Nupeko Forest(28). Neutralizing antibodies for DEN-2 antigen were commonly found in the sera of village inhabitants as well as in some monkey sera from an area where A. aegypti is not prevalent(29). Further investigations were carried out in the southern part of Nigeria by means of a serological survey in monkeys and humans(30), and the results showed that DEN viruses were prevalent in the four ecological zones studied. In the rain forest area the antibody prevalence was about equal in urban and rural areas (43 per cent and 41 per cent respectively). The highest prevalence was in derived savannah zones, and it was higher in urban than in rural areas (67 per cent and 33 per cent respectively). In the southern Guinea savannah zone, the rate was also higher in urban areas but lower than in derived savannah zones (urban areas 45 per cent and rural areas 32 per cent). On the plateau, dengue antibodies were found only in the urban community (30 per cent). The hypothesis of a sylvatic cycle of DEN viruses was reinforced by the isolation of a DEN-2 virus strain from wild caught unidentified Stegomyia, probably Aedes luteocephalus, on the Jos Plateau in November 1969, and by the isolation of two strains of DEN-1 virus from Aedes africanus in the Mamu River Forest (Eastern Nigeria) in 1977(31).

Burkina Faso (formerly Upper Volta)

During a longitudinal survey of yellow fever virus circulation in different wooded areas of the eastern part of Upper Volta, a total of 68 strains of DEN-2 virus were isolated between September and November 1980, at the end of the rainy season and the beginning of the dry season(32,33). The main mosquito involved was Aedes (Stegomyia) luteocephalus, from which 65 strains were isolated, followed by Aedes (Stegomyia) africanus, which gave rise to two strains of DEN-2. Aedes (Aedimorphus) cummins gave rise to one strain of DEN-2, but this isolate was not thought to necessarily signify that this species of Aedes is an efficient vector. No significant differences were observed in the infection rates of A. luteocephalus during the three months of the survey, but significant differences were noticed according to the type of wooded area.

No serological data are available for this epizootic, but, bearing in mind the possible role of unknown vertebrates, it is likely that monkeys and probably humans played the main role as vertebrate hosts.

After this epizootic, one strain of DEN-2 virus was isolated from A. aegypti caught in the town of Bobo-Dioulasso and there was one case of disease in an expatriate which was serologically confirmed. At the same time, an epidemic occurred in the town of Ouagadougou, where 29 expatriates and one African showed an intense dengue
syndrome\(^{(39)}\). DEN-2 virus was isolated from six cases, and serological investigation produced evidence for the dengue origin of the disease in 21 cases. Only two cases were children. No DHF or DSS were recorded, but there were slight haemorrhagic cutaneous symptoms in four cases. This epidemic occurred between September and December, in the late rainy season and early dry season. Seasonal epidemics of dengue-like fever were noticed for at least five consecutive years in Ouagadougou, but DEN virus was isolated only in 1982. In 1983, no DEN isolate was obtained from febrile patients.

Ivory Coast

As in Upper Volta, an important sylvatic circulation of DEN-2 virus was found in 1980 in the sub-Saharan savannas\(^{(33,35)}\). Twenty-eight strains were isolated from wild vectors: *Aedes (Diceromyia) furcifertaylori* group (17 strains), *Aedes (Stegomyia) luteocephalus* (seven strains), *Aedes (Stegomyia) opok* (three strains) and *Aedes (Stegomyia) africanus* (one strain); one strain originated from wild caught males of the *Aedes furcifertaylori* group. The first isolate was made in May, after about one month of the rainy season, and the last was made in October in the late rainy season.

A serological survey carried out in humans in the same area one year later showed that HI antibodies were present for one or several Flavivirus antigens, but that the dengue antibodies were always at a very low titre\(^{(33)}\). For this reason it was thought that monkeys were the main vertebrate hosts involved in the transmission.

A single human case of DEN-2 infection was diagnosed in the town of Abidjan in 1982, two years after the epizootic.

More recently\(^{(42)}\), ten new isolates of DEN-2 virus were obtained in 1985, 1986 and 1987 from a savannah area, from sylvatic vectors (one from *A. africanus*, six from *A. luteocephalus*) as well as from domestic ones (three from *A. aegypti*). No human repercussion was noticed during this period, but this is evidence that the virus is still present in the country.

In 1985, one single strain of DEN-1 was isolated from domestic *A. aegypti* caught in a savannah village\(^{(42)}\).

Senegal

Three types of DEN virus have been isolated in Senegal and observations have varied according to the virus type involved.

Dengue 1

In the village of Bandia, 80 km south-east of Dakar, the population was under surveillance for arbovirus circulation and sera were collected from every febrile patient. In October 1979 two strains of DEN-1 virus were isolated from the sera of Senegalese patients with very mild diseases. At the same time two more cases were serologically diagnosed in Europeans who slept in the bush in the same area; these were more severe diseases, but without haemorrhagic or shock syndromes. Serological surveillance was maintained in this area from 1980 to 1982, when 21.5 per cent of children had DEN-1 antibodies but all the results were negative for sera of children born after the outbreak.

Sera were also available from the region of Kaolack (120 km east-south-east of Bandia), where children were under surveillance for another purpose and sera were collected every three months between 1978 and 1983. Twenty-one children were serologically tested for DEN-1 antigen and nine showed a seroconversion between September 1979 and January 1980 (unpublished data); five of these were demonstrated by the CF test and four by the ELISA test.

In 1981 a small outbreak of yellow fever occurred around the small town of Mekhe (75 km north-east of Bandia); a high prevalence of DEN-1 antibodies was found in the sera of two year old children. The same observations were made in 1982 (unpublished data) during a chikungunya outbreak in the area of Touba (130 km east of Bandia).

Thus, there was an extensive epidemic of DEN-1 in western Senegal at the end of 1979 in the late rainy season and early dry season. Diseases were
so mild that this outbreak was unnoticed by the Public Health Authorities. A retrospective study of human and monkey sera collected in the area of eastern Senegal (a zone known for its sylvatic circulation of arboviruses) did not show any trace of DEN-1 virus circulation. It is not known where the virus came from, but, in the absence of a sylvatic circulation, it was thought that the virus could have been introduced through the harbour or the airport of Dakar.

**Dengue 2**

A single isolate was made in the same village of Bandia in late 1970, but the definite identification of the virus only occurred in 1977 and no serological investigation could be carried out. In November 1974 a second isolate was made from a pool of *A. luteocephalus* caught in a non-human environment in Eastern Senegal and a retrospective study of monkeys caught in 1974-1975 showed evidence of a small epizootic of DEN-2 in 1974. The two strains isolated were obtained following inoculation into suckling mice. From 1979 onwards DEN virus studies became a priority and more sensitive inoculation techniques were introduced, namely inoculation in *Toxorhynchites* or in insect cell culture.

In 1981, a large epizootic occurred in Eastern Senegal. Two hundred and thirteen DEN-2 virus strains were isolated from mosquitoes living in jungle areas and one strain was isolated from the blood of a free-living red monkey, *Erythrocebus patas*. Only three species of mosquito gave rise to virus isolates: *Aedes* (*Diceromyia*) furcifer (68 strains), *Aedes* (*Diceromyia*) taylori (37 strains) and *Aedes* (*Stegomyia*) luteocephalus (81 strains). An additional strain was isolated from a pool of *male Aedes taylori*. The first isolate was made in the early rainy season in June, and the peak time for isolation occurred in September-October. The epizootic decreased abruptly in November and December, at the beginning of the dry season. The infected monkey was caught in September. The early isolations of June and July (especially that from male mosquitoes) point to the conclusion that the virus was already present in late 1980. Despite this intense epizootic, three other flaviviruses were also isolated in 1981: Zika, Wesselsbron and Kedougou. A small resurgence of the epizootic was observed in 1982, when a single strain was isolated in September from *A. luteocephalus*. In November 1981, four pools of potential vectors caught in the neighbouring area of Guinea yielded four virus strains; three from *A. africanus* and one from *A. luteocephalus*.

The repercussion in human terms of this epizootic seemed to be very low; in January 1983 a retrospective survey was carried out in 13 health centres in the area, but it did not allow evaluation of the degree of human infection, most of the febrile diseases being attributed to malaria. In any case, no trace of DHF or DSS could be found. A single case of DEN-2 infection was observed in a European member of the entomological team; the infection was severe, with a typical rash, and a residual pain in the shoulder joint which persisted for one year. Serological surveillance of children is continuing, and results have indicated that 11 per cent of children were in contact with the virus in 1981. More interesting is the fact that isolated positive responses to DEN-2 antigen were found every year from 1982 to 1985, especially in 1984 when three per cent of the tested sera contained CF antibodies.

Serological studies in monkeys showed results identical to those of the virological study, and from June to December 1981, 27 per cent of monkeys had CF antibodies, often at high titres. In 1982, 33 per cent were positive but at a lower titre, which was attributed to a cumulative effect. Results were entirely negative in 1983 and 1984, but a positive response for IgM using the ELISA test was found in December 1985, showing that the virus was still present in the area.

After this large epizootic, an epidemic was expected in the western part of Senegal, as is usual with yellow fever virus. Despite special surveillance, no epidemic occurred and a single case was diagnosed in a European who travelled in the south of the country in November 1983. There is a suspicion that the virus migrated to the West, as...
yellow fever virus usually does. It is not understood, however, why there has been no epidemic, despite the lack of vaccine.

Dengue 4
DEN-4 virus was first isolated in December 1981 from a patient who had just arrived from the Caribbean Region (Haiti) and who fell ill in Dakar.

In November 1983, a small epidemic was observed in a European family who lived in Dakar and who did not leave the town in the 14 days before they fell ill. The two children were first infected, but no virological or serological investigations were performed; after about one week, the father and the mother had fever, headache and myalgia, and one of them developed rashes; DEN-4 virus was isolated from both. A third case was serologically diagnosed in Casamance (South-West Senegal). These cases were the first evidence of DEN-4 transmission in Africa. Serological investigations in eastern Senegal provided no evidence of DEN-4 virus circulation, either in humans or in monkeys.

More investigations are needed to establish the origin of the virus. The Dakar observations indicate that the virus may have been introduced, but the Casamance case could have originated from a local sylvatic cycle.

5.3 Epidemiology

Our knowledge of dengue in Africa is still too fragmentary to give a clear picture of its epidemiology. The situation seems to be different, not only between East and West Africa, but also according to the virus type.

Geographic distribution
Dengue is actually known only from the coastal areas and islands of East and South Africa and from most of West Africa. There is no record of the disease from Central Africa, although it is not known whether this is due to the lack of investigations or to the results of investigations being negative.

In South and East Africa, dengue seems to be essentially urban, whereas in West Africa circulation occurs as well in rural areas. This is probably related to the species of vector involved, and their preferences for rural and/or urban environments.

Annual and seasonal distribution
Here again there is a difference between East and West Africa. In East Africa cases have been recorded during epidemics lasting several years (Seychelles Islands), until a high percentage of the population has acquired immunity. In West Africa circulation seems to be permanent, at least for DEN-1 and DEN-2 viruses (Nigeria, Senegal), with episodic urban or sylvatic outbreaks.

Seasonally, the pattern of cases depends upon the climatic conditions and the type of vector involved. When the transmission is urban, cases may occur all the year round, with a peak during the rainy season when the vector is abundant. In humid areas of West Africa, cases may also be observed at any time (Nigeria), but in savannah areas they generally occur at the end of the rainy season and the beginning of the dry season (Burkina Faso, Senegal).

Age distribution
Most of the diagnosed cases have been children, but this could be due to the fact that the surveys have been focused upon children. The diagnosis is easier in this group of the population for the reason that they are less often in contact with other flaviviruses.

Clinical features
Clinical descriptions of the diseases are relatively few in the literature. Most of the recorded cases have been mild infections with a typical dengue syndrome involving fever, asthenia, generalized pain, and often rashes which are characteristic of the disease and in which the erythematous macules are slightly elevated and there is furfuraceous and
pruriginous desquamation. In other arboviruses, such as chikungunya, eruption is generally flat and there is no desquamation. Vomiting and diarrhoea have often been recorded after a few days of the disease, as well as some upper respiratory tract troubles (cough, rhinorrhea). The duration is generally three to five days, but the weakness may last several weeks; joint pains may also persist as in chikungunya.

In the Seychelles Islands and in Kenya, DEN virus was suspected to have a role in the increase in deaths caused by viral encephalitis. In Nigeria, three cases with febrile convulsions were reported in children.

Haemorrhagic and shock syndromes seem to be rare in Africa. The only record of these types of dengue was from South Africa in 1926-1927, perhaps also from Mozambique in 1985 when two deaths were reported.

The disease generally appears to be more serious in East Africa than in West Africa. In this latter area, with the exception of Nigeria, most of the cases reported have been in expatriates of European origin who were more seriously affected than African people. This can be explained by the fact that Europeans more often visit the doctor when they feel ill, but, in fact, not very much is known about the clinical aspect of the disease in Africans. It is only suspected that infections are very mild since such extensive epidemics as the Senegalese DEN-1 epidemic of 1979 was unnoticed by health authorities. This aspect of dengue in West Africa needs further investigation.

Laboratory findings

Most of the strains that have been isolated were isolated by intra-cerebral inoculation into suckling mice, a technique which is not very sensitive. In Senegal, intra-thoracic inoculation in Toxorhynchites brevipalpis was used during the 1981 epizootic, and this technique is probably responsible for the greater number of strains obtained in Senegal than in Burkina Faso and the Ivory Coast. Further, comparison of different techniques has shown that the most sensitive and fast technique for isolating sylvatic strains of DEN virus is that of inoculation into Aedes pseudoscutellaris cell lines (Mos 61). There is no toxic effect from blood or organ samples as is often seen in Toxorhynchites, and the diagnosis, using monoclonal antibodies in an indirect fluorescent assay, is faster (four to seven days).

Transmission cycles

The single observations of DEN-3 in Mozambique and of DEN-4 in Senegal did not allow any epidemiological conclusions, except that they occurred in urban environments. There is a clear distinction between the observations made in East and West Africa for DEN-1 and DEN-2 viruses.

East and South Africa

All the records from these regions concern typical urban transmission and involve domestic (A. aegyptii) or peri-domestic (A. albopictus) vectors. There is no record of sylvatic transmission, although investigations in this field are few. The fact that all records have come from coastal areas or from islands and that there are commercial and cultural relations with South-East Asia lead to the conclusion that DEN viruses might be periodically introduced into this region from South-East Asia, and that epidemics occur where vectors are abundant enough. Epidemiologically, East Africa seems to be closer to South-East Asia than to West Africa.

West Africa

Much information has been collected on the transmission of DEN viruses in West Africa during the past 20 years and conclusions are different for type 1 and 2 viruses.

DEN-1 is known only from Nigeria and Senegal. Cases, which have been sporadic in Nigeria and epidemic in Senegal, have occurred only in urban environments and there is no evidence of sylvatic circulation. Two strains of DEN-1 have been isolated from A. africanus in Eastern Nigeria, where this mosquito is known to act as a domestic vector, biting inside the villages and even inside dwell-
ings\(^{(26)}\). No trace of sylvatic circulation of DEN-1 was observed in Eastern Senegal where DEN-2 sylvatic transmission seems to be permanent.

These observations led to the hypothesis that DEN-1 is endemic in humans in West Africa, and that man might be the only vertebrate host involved in the cycle. However, this hypothesis needs further research because it is not impossible that DEN-1 virus has been introduced into Nigeria and Senegal.

There is a lot of information concerning DEN-2 virus. Sylvatic circulation has been demonstrated wherever it has been looked for, both by serological survey as in Nigeria and by virus isolation as in many other countries (Burkina Faso, Ivory Coast, Senegal and Guinea).

The sylvatic cycle of DEN-2 virus seems to be very close to that of the yellow fever virus, which has been the subject of intensive studies in West Africa for a number of years. The main vectors are the same: *Aedes* (*Diceromyia*) furcifer and taylori, and *Aedes* (*Stegomyia*) africanus lutoecephalus and opok. The only wild vertebrate hosts actually involved are monkeys (and galagos in Nigeria). Despite these similarities, the dynamics of the epizootics are somewhat different; dengue epizootics are more explosive, shorter in duration, and reach higher levels of incidence.

As far as yellow fever virus is concerned, sylvatic circulation occurs in rain forest areas where it seems to be enzootic, although the main sylvatic cycles occur in humid and semi-humid savannahs where DEN-2 is epizootic within an enzootic background. In Senegal the presence of the virus has been demonstrated for six consecutive years.

The only information we have on the periodicity of epizootics of DEN-2 comes from Senegal, where a small epizootic was recorded in 1974 and a large epizootic in 1980. In this area, where monkeys are very abundant, yellow fever epizootics are observed every four or five years; and their intensity increases with the progressive decrease in immunity of monkeys, so that big epizootics occur every 13 to 14 years. In other countries where monkeys are less abundant, epizootics have the same periodicity, but there is no variation in the intensity. Thus, it appears that the periodicity of DEN-2 epizootics is similar to that of yellow fever epizootics.

Maintenance of the virus is probably due to transovarial transmission occurring in the vectors, as shown by the two isolates from wild caught male *Diceromyia* in the Ivory Coast and Senegal. Thus, mosquitoes would act as vectors as well as virus reservoirs; monkeys would be only periodic amplifying hosts.

A big difference between DEN-2 and yellow fever virus concerns the potentiality for producing epidemics, which appears to be much lower for DEN-2. Yellow fever epidemics generally occur at the same time as epizootics (Burkina Faso, 1983) or a few years later (Senegal, 1976-1981). After the intense sylvatic circulation of DEN-2 virus in 1980-1981, human infections were relatively few: a single case in western Burkina Faso, a small epidemic in eastern Burkina Faso, a single case in the Ivory Coast and a single case in Senegal. Most of these cases were in Europeans and they occurred two years after the local epizootics.

Whether DEN-2 virus originated in Africa or was introduced from other regions has not been elucidated. The only thing certain is that, if it was introduced, this happened many years ago, as the virus is now well adapted to the West African ecosystem.

**References**


42. R. Cordelletier. Farnmental Communication.
Chapter 2

Clinical Manifestations of Dengue/Dengue Haemorrhagic Fever

by

Suchitra Nimmanitiya

1. INTRODUCTION

Dengue virus infection may be asymptomatic or may lead to undifferentiated febrile illness (viral syndrome), dengue fever (DF), or dengue haemorrhagic fever (DHF) depending largely on age and immunological conditions.

Undifferentiated Fever

Infants and children who have been infected with dengue virus for the first time (i.e. primary dengue infection) will develop simple fever indistinguishable from other viral infections. Maculopapular rashes may accompany the fever or may appear during defervescence.

Figure 1. Manifestations of the dengue syndrome

![Diagram of dengue manifestations]
**Dengue Fever**

Dengue fever is most common in adults and older children. It is an acute biphasic fever with headache, myalgia, arthralgia, rashes and leucopenia. Although DF is commonly benign, it may be an incapacitating disease with severe muscle and joint pain (break-bone fever), particularly in adults, and occasionally with unusual haemorrhage. Infection with one dengue serotype gives solid immunity to that particular serotype but only partial protection against the others. In dengue endemic areas, DF seldom occurs among indigenous people.

**Dengue Haemorrhagic Fever**

Dengue haemorrhagic fever is most common in children less than 15 years of age and causes a significant number of deaths. DHF is characterized by acute fever associated with haemorrhagic diathesis and a tendency to develop fatal shock (dengue shock syndrome). Abnormal haemostasis and plasma leakage are the main pathophysiological changes, with thrombocytopenia and haemoconcentration presenting as constant findings. DHF occurs most commonly in children who have experienced a primary dengue infection.

**2. DENGUE FEVER**

**Clinical Course and Clinical Laboratory Findings**

After an incubation period of four to six days (minimum three and maximum ten days) from the time of the mosquito bite, various nonspecific undifferentiated symptoms, such as headache, backache, stiffness, general malaise and flushed or mottled facial skin may develop. Typically the onset is sudden with a sharp rise of temperature associated with severe headache and sometimes chilliness.

Within 24 hours, the following symptoms develop: retro-orbital pain particularly on eye movement or eye pressure, photophobia, backache and pain in the muscle and joints of the extremities. Other common symptoms include: extreme weakness, anorexia, constipation, altered taste sensation, colicky pain and abdominal tenderness, dragging pain in the inguinal region, sore throat and general depression. These symptoms vary in severity and duration.

Fever: the temperature is usually between 39°C and 40°C, and the fever lasts approximately six days. Occasionally a biphasic fever curve is observed.

Rashes: diffuse flushing, mottling or fleeting pin-point eruptions may be observed on the face, neck and chest during the first half of the febrile period and a conspicuous rash, that may be maculopapular or scarlatiniform, appears on approximately the third or fourth day. This rash starts on the chest and trunk and spreads to the extremities and face and may be accompanied by itching and dermal hyperaesthesia. Towards the end of the febrile period or immediately after defervescence the generalized rash fades and localized clusters of petechiae may appear over the dorsum of the feet, and on the legs, hands and arms. This rash is characterized by scattered pale round areas of normal skin.

![Rashes in dengue fever](image)
There may be generalized enlargement of the lymph nodes but the liver and spleen are not usually enlarged.

Course: the relative duration or severity of DF varies between individuals in a given epidemic, as well as from one epidemic to another.

Convalescence may be abrupt and uneventful but is often prolonged, sometimes taking several weeks, and may be accompanied by pronounced asthenia and depression. Bradycardia is common during this period.

Haemorrhagic Manifestations: Skin haemorrhage with a positive tourniquet test and/or petechiae are not uncommon. There have been reports of epistaxis, gingival bleeding, haematuria and hypermenorrhoea in many epidemics of DF, and on rare occasions severe bleeding has caused death in some epidemics. DF complicated by unusual haemorrhage must be differentiated from DHF.

The most significant laboratory findings during the acute illness concern the changes in the total white blood cells (WBC) and the differential count. As a rule, the total WBC count is usually normal at the onset of fever but then leukopenia develops and lasts throughout the febrile period. Initially, there is a progressive shift of neutrophils to the left, that is, an increase of immature nonsegmented nuclear (band) forms which persist into convalescence, and there is marked depletion of circulating lymphocytes at the beginning. Platelet counts are usually normal as are other components of the blood clotting mechanism. On rare occasions however, mild thrombocytopenia may occur in DF[3]. Some biochemical parameters and liver enzymes are within the normal ranges.

Differential Diagnosis

Differential diagnosis of DF includes a wide variety of viral (including chikungunya), bacterial and rickettsial infections that produce a similar syndrome. It is impossible to diagnose mild dengue infection clinically, particularly in sporadic cases.

Diagnosis

Etiologic confirmation can be made by serologic study of dengue antibody response and virus isolation.

3. DENGUE HAEMORRHAGIC FEVER[1,3,4]

3.1 Clinical Course

Typical DHF is an acute illness of children characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, hepatomegaly, and often circulatory failure. The major pathophysiological changes that determine the severity of disease in DHF and differentiate it from DF are plasma leakage and abnormal haemostasis, as manifested by a rising haematocrit value and moderate to marked thrombocytopenia. These two clinical laboratory changes are distinctive and constant findings in DHF.

Following an incubation period of four to six days (thought to be the same as in DF), the illness commonly begins abruptly with high fever accompanied by facial flushing and headache. Anorexia, vomiting, epigastric discomfort, tenderness at the right costal margin and generalized abdominal pain are common. During the first few days the illness resembles classical DF, but maculopapular rash, usually rubelliform type, is less common. It may appear early or late in the course of the illness. Occasionally, the temperature may be 40°C – 41°C and febrile convulsion may occur particularly in infants.

A haemorrhagic diathesis is commonly demonstrated by scattered fine petechiae on the extremities, axillae, trunk and face in the early febrile phase. A positive tourniquet test and a tendency to bruise at venepuncture sites are always present. Bleeding from nose, gum and gastro-intestinal tract are less common but can be severe. Massive gastro-intestinal bleeding is often associated with prolonged shock. Gross haematuria is extremely rare.

The critical stage is reached after two to seven days when the fever subsides. Accompanying or shortly after a rapid drop in body temperature, varying degrees of circulatory disturbance occur. The child is commonly sweating, restless and has cool extremities.
Clinical Manifestations of Dengue/DHF

Figure 3. The spectrum of dengue diseases

In less severe cases, the changes in vital signs are minimal and transient and the patient recovers spontaneously or after a brief period of therapy.

Table 1. Non-specific constitutional symptoms observed in haemorrhagic fever patients with dengue and chikungunya infection (modified from Nimmannitya et al. 1969)

<table>
<thead>
<tr>
<th>Findings</th>
<th>Percentage of patients showing symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dengue</td>
</tr>
<tr>
<td>Cough</td>
<td>21.5%</td>
</tr>
<tr>
<td>Restlessness</td>
<td>21.5%</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>21.5%</td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>12.0%</td>
</tr>
<tr>
<td>Myalgia/Arthralgia</td>
<td>12.0%</td>
</tr>
<tr>
<td>Exanthem</td>
<td>3.3%</td>
</tr>
<tr>
<td>Abnormal reflex</td>
<td>6.7%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6.1%</td>
</tr>
<tr>
<td>Palpable spleen</td>
<td>6.3%</td>
</tr>
<tr>
<td>Coma</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

*Statistically significant difference
*Infants under 6 months

Table 2. Constitutional symptoms observed in haemorrhagic fever patients with dengue and chikungunya infection (modified from Nimmannitya et al. 1969)

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Percentage of patients showing symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever - duration</td>
<td></td>
</tr>
<tr>
<td>2-4 days</td>
<td>23.6%</td>
</tr>
<tr>
<td>5-7 days</td>
<td>59.0%</td>
</tr>
<tr>
<td>more than 7 days</td>
<td>17.4%</td>
</tr>
<tr>
<td>Haemorrhagic manifestations</td>
<td></td>
</tr>
<tr>
<td>positive tourniquet test</td>
<td>53.9%</td>
</tr>
<tr>
<td>petechiae scattered</td>
<td>40.5%</td>
</tr>
<tr>
<td>Petechial rash (confluent)</td>
<td>10.1%</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>10.9%</td>
</tr>
<tr>
<td>Gum bleeding</td>
<td>1.5%</td>
</tr>
<tr>
<td>Melena/hematemesis</td>
<td>11.8%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>90.9%</td>
</tr>
<tr>
<td>Shock</td>
<td>35.2%</td>
</tr>
</tbody>
</table>

In more severe cases, the patient's condition rapidly deteriorates as shock ensues. Acute abdominal pain is a frequent complaint shortly before onset of shock. The patient becomes restless and circumoral cyanosis is more prominent. The skin is cold, clammy, purplish and blotchy. The pulse is rapid and feeble, while a narrowing pulse pressure...
of less than 20 mm Hg (e.g. 100/80, 100/90 mm Hg) is usually observed in the early stages of shock. Shock progresses rapidly into profound shock, with unobtainable blood pressure and/or pulse, and the patient may die within 24 hours. Prolonged shock is often complicated by metabolic acidosis and severe bleeding, which indicates a poor prognosis. If the patient is appropriately treated, however, before irreversible shock has developed, rapid recovery is the rule.

Encephalitic signs associated with intracranial haemorrhage, and metabolic and electrolyte disturbances may occur but are uncommon; they indicate a grave prognosis.

Course: convalescence is commonly short and uneventful, and may be accompanied by sinus bradycardia and a characteristic confluent petechial rash with scattered round areas of pale skin as described in DF. The duration of DHF is between seven and ten days in most cases.

### 3.2 Clinical Laboratory Findings

- A normal WBC or leucopenia is common initially, with neutrophils predominating.

- Towards the end of the febrile phase, a relative lymphocytosis with more than 15 per cent atypical lymphocytes is common.

- Thrombocytopenia and haemoconcentration are constant findings. The platelet count drops shortly before or at the same time as the haematocrit value increases; both changes occur before the subsidence of fever and shock.

- Clotting abnormalities are common and appear to correlate with disease severity.

- Hypoproteinaemia, particularly hypoalbuminaemia, is common.

- There is hyponatraemia in severe cases.

- Serum alanine aminotransferase (ALT) is slightly elevated.

Clinical diagnosis of DHF can be made with 90-95 per cent confidence (Children's Hospital, Bangkok) using the following criteria which are based on the presence of major manifestations and the course of illness, which is stereotyped.
Clinical Criteria

- Fever - acute onset, high, continuous, and lasting two to seven days in most cases.
- Haemorrhagic manifestations, including at least a positive tourniquet test and any of the following: scattered petechiae, confluent petechial rash, epistaxis, gum bleeding, and/or melena/haematemesis.
- Enlargement of the liver.
- Shock - manifested by rapid and weak pulse with narrowing of the pulse pressure (20 mm Hg or less) or hypotension, with the presence of cold clammy skin and restlessness.

Laboratory Criteria

- Thrombocytopenia (more than 100,000/mm³).
- Haemoconcentration: haematocrit increased by 20 per cent or more of baseline value.

The presence of petechiae, a positive tourniquet test or easy bruising in a patient with high fever suggest dengue infection. As the disease progresses, an enlargement of the liver, which is usually soft and tender, gives more support for clinical diagnosis. The diagnosis of DHF becomes certain when the platelet count drop shortly before, or simultaneously with, a rise in haematocrit value. The time course relationship between the drop in platelet count and the rapid rise in haematocrit value appears to be unique in DHF. These changes are correlated with disease severity as they represent the pathophysiological hallmark, viz. abnormal haemostasis and plasma leakage. When there is anaemia or severe haemorrhage, pleural effusion (more often on the right side by chest X-ray) and/or hypoalbuminaemia provide supporting evidence of plasma leakage.

3.3 Grading of Severity of DHF(1,4)

The severity of DHF has been classified into four grades according to two pathophysiological hallmarks - shock and bleeding.

Grade I
Fever accompanied by non-specific constitutional symptoms. The only haemorrhagic manifestation is a positive tourniquet test.

Grade II
Patient with spontaneous bleeding usually in the form of skin and/or other haemorrhages in addition to the manifestations in Grade I.

Grade III
Circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mm Hg or less) or hypotension with the presence of cold clammy skin and restlessness.

Grade IV
Profound shock with undetectable blood pressure and pulse.

Figure 5. Typical clinical course of dengue haemorrhagic fever in a nine-year-old girl (with secondary dengue infection)
The presence of thrombocytopenia with concurrent haemoconcentration differentiates grade I and grade II DHF from DF and other diseases.

Early in the febrile phase, the differential diagnosis includes a wide spectrum of viral and bacterial infections. By the third or fourth day, usually before shock occurs, a drop in platelet count and a rise in the haematocrit value help in establishing the diagnosis. When shock develops with other manifestations and two essential laboratory findings, thrombocytopenia with concurrent haemoconcentration, the diagnosis of DHF (DSS) is most certain. Other evidence of plasma leakage, including pleural effusion, ascites, and hypoproteinaemia, differentiates dengue shock syndrome (DSS) from endotoxic shock.

3.4 Dengue Infection with Unusual Manifestation(1,5)

General vital organs are not primarily involved in DHF. Signs and symptoms of organ involvement are usually secondary to plasma leakage, shock and haemorrhage. Some patients with DF and DHF present unusual manifestations; and central nervous system (CNS) involvement may be manifested as convulsion, spasticity and/or change of consciousness. Hepatic dysfunction and renal failure may also occur.

Central nervous system involvement

CNS involvement has been shown to be encephalopathy (not encephalitis, but with normal cerebrospinal fluid (CSF) and no pathological changes of inflammation) associated with hepatic failure in the majority of cases. Reye's syndrome associated with dengue infection has been demonstrated. Transient paresis has been observed in some epidemics.

Hepatic involvement

Hepatic dysfunction presents with jaundice and markedly elevated serum alanine aminotransferase (ALT). Some manifestations are due to drugs (including paracetamol, salicylate) or toxic substances. There is some evidence for pre-existing liver damage or concurrent infections with other viruses e.g. hepatitis B virus (HBV).

Unusual manifestations with encephalopathy and hepatic dysfunction are observed more frequently in infants under the age of 12 months.

Renal failure

Following prolonged shock with inappropriate management, haemolytic uraemic syndrome has been reported associated with DHF in patients with abnormal red blood cell enzymes (glucose-6-phosphate dehydrogenase deficiency).

References

Chapter 3
Management of Dengue and Dengue Haemorrhagic Fever
by
Suchitra Nimmannitya

1. DENGUE FEVER

The management of dengue fever (DF) is symptomatic and supportive.

- Bed rest is advisable during the acute febrile phase.
- Antipyretics or sponging are required to keep the body temperature below 40°C. Aspirin should be avoided, particularly in areas where dengue haemorrhagic fever (DHF) is endemic, since it may cause gastritis, bleeding and acidosis.
- Analgesics or mild sedatives may be required for those with severe pain.
- Oral fluid and electrolyte therapy is recommended for patients with excessive sweating, vomiting or diarrhoea.

In areas which are endemic for dengue haemorrhagic fever (DHF), patients with dengue syndrome should be monitored until they become afebrile and platelet counts and hematocrit determinations are normal.

2. DENGUE HAEMORRHRAGIC FEVER

2.1 General Considerations

The major pathophysiological abnormality seen in dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) is an acute increase in vascular permeability that leads to leakage of plasma and subsequently to hypovolaemic shock if the loss of plasma is critical. Plasma volume studies reveal a reduction of more than 20% in severe cases. Supportive evidence for plasma leakage includes: serous effusion, ascites, haemoconcentration and hypoproteinaemia. In severe cases, the onset of shock is acute, hematocrit readings rise sharply and plasma escapes through the endothelium. Hypovolaemic shock leads to tissue anoxia, metabolic acidosis and death if uncorrected. The acute onset of shock and the rapid, often dramatic, recovery when properly treated together with the fact that no destructive or inflammatory vascular lesions are observed and that there are no sequelae, suggest transient functional, vascular permeability changes that may be due to short acting pharmacological mediators. C3a and C5a anaphylatoxins, cleavage products of
complement activation, are elevated around the time of leakage, which indicates an active role of these anaphylatoxins in the pathogenesis of increased vascular permeability.

Haemostatic changes in DHF involve three factors: vasculopathy, thrombocytopenia and coagulopathy. All patients demonstrate an increase in vascular fragility (positive tourniquet test) and moderate to marked thrombocytopenia. About 80 per cent of patients with DSS and 17 per cent of non-shock cases have an abnormal coagulogram as evidenced by concomitant thrombocytopenia, prolonged partial thromboplastin time (PTT), decreased fibrinogen levels, and increased fibrinogen degradation products (FDP), suggesting disseminated intravascular clotting (DIC). In the case of prolonged uncontrolled shock, DIC may cause important clinical bleeding and may play an important part in the development of lethal shock. About one third of shock cases, mostly those with refractory shock, present with bleeding, mainly from the gastrointestinal tract. Gastro-intestinal haemorrhage is a fairly constant finding at autopsy in the majority of patients who die.

Early and effective replacement of plasma loss with plasma, plasma expander and/or fluid and electrolyte solution, results in a favourable outcome in most cases. With adequate volume replacement, DSS is rapidly reversible. Early recognition of shock and rapid volume replacement will usually prevent clinical DIC. Prognosis depends upon early monitoring of patients for a drop in platelet count and rise in haematocrit reading. Early volume replacement, as haematocrit values rise sharply from the plasma leakage, can prevent the development of shock. Serial determinations of platelet and haematocrit values are essential for early recognition and prevention of shock. The critical period in severe cases is the transition from the febrile to the afebrile phase, which usually occurs after approximately the third day.

2.2 Treatment Regimen

The management of DHF during the febrile phase is similar to that of DF, but antipyretics should be used with caution. Salicylates should be avoided since they may cause bleeding and acidosis.

Oral electrolyte solution (as used in diarrhoea) or fruit juice is recommended during the febrile phase.

A rise in haematocrit value of more than 20 per cent from baseline indicates significant plasma loss and a need for parenteral fluid therapy. In mild and moderate cases (Grades I and II), volume replacement can be given in an out-patient department rehydration unit for a period of 12-24 hours.

2.3 The Need for Hospitalization

Patients who are restless and who have cool extremities, acute abdominal pain and oliguria should be admitted to hospital. Patients with any signs of bleeding and persistently high haematocrit values, despite being given volume replacement, should be promptly admitted to hospital.

2.4 Volume Replacement in Dengue Haemorrhagic Fever and Dengue Shock Syndrome

The volume and type of fluid should be similar to that used in the treatment of diarrhoea with moderate isotonic dehydration (six to ten per cent deficit), but the rate should be carefully titrated. The required fluid volume should be charted on a two to three hourly basis and the rate of administration adjusted throughout the 24-48 hour period of leakage. Serial haematocrit determinations, every four to six hours, and frequent recording of vital signs are recommended for adjusting the fluid replacement in order to assure adequate volume replacement and avoid over-transfusion.

Written orders should be explicit as to the type of solution and the rate of administration.

A rough estimate of flow may be derived from the formula:

\[ \text{ml/hour} = \text{(drop/min)} \times 3. \]
The fluid replacement should be the minimum volume that is sufficient to maintain effective circulation during the period of leakage (24-48 hours). Excessive replacement will cause respiratory distress (from massive pleural effusion and ascites), pulmonary congestion and oedema.

**Type of fluid:**

- **Crystallloid:**

  Five per cent dextrose in lactated Ringer’s solution (five per cent D/RL)

  Five per cent dextrose in acetated Ringer’s solution (five per cent D/RA)

  Five per cent dextrose in half strength normal saline solution (five per cent D/1/2/NSS)

  Five per cent dextrose in normal saline solution (five per cent D/NSS)

- **Colloidal:**

  Dextran 40
  Plasma

**An example of treatment:**

The patient: A child with DHF grade II. A two year old boy, of ten kg body weight and with a history of high fever for four days and vomiting.

- **Physical Examination shows:**

  Temperature 37°C;
  Pulse rate 120/min;
  Blood Pressure 100/70 mmHg;
  Tourniquet Test: Positive;
  Petechiae: Positive; and
  Liver two cm below costal margin and tender.

- **Clinical Laboratory examination shows:**

  Plaeter: 0-1/OF (estimate, from peripheral blood smear, of the average of 10 oil field (OF) counts);
**Haematocrit:** 45 per cent (35 per cent on first admission)

- **Volume Therapy in DHF grade II:**

  Calculate the amount of IV fluid as moderate dehydration (six to ten per cent deficit)

  \[= 1600 \text{ ml/day or } 67 \text{ ml/h} \]
  \[= 6.7 \text{ ml/kg/h or } 22 \text{ drop/min} \]

  (*calculated as six per cent deficit of fluid from the table below.*)

  Type of fluid should be isotonic five per cent D/RL or five per cent D/NSS

  Order 500 ml of five per cent D/RL

  Start at the rate of seven ml/kg/hr or 22 drop/min

  Adjust the rate of IV fluid according to vital signs and haematocrit values as shown in Figure 2.

  **Table: Fluids for moderate dehydration**

<table>
<thead>
<tr>
<th>Weight on admission</th>
<th>7 kg</th>
<th>7-11 kg</th>
<th>11-16 kg</th>
<th>16 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day*</td>
<td>220</td>
<td>165</td>
<td>122</td>
<td>88</td>
</tr>
<tr>
<td>Second day*</td>
<td>165</td>
<td>132</td>
<td>88</td>
<td>68</td>
</tr>
<tr>
<td>Third day*</td>
<td>132</td>
<td>56</td>
<td>88</td>
<td>68</td>
</tr>
</tbody>
</table>

*In DHF on the first day that haematocrit values rise more than 20 per cent, the amount of fluid should not exceed 500 ml per order or should not be given for a period longer than six hours. (Adapted from Pediatric Clinic of North America 1964, 11: 1093.)*

**2.5 Management of Shock**

DSS is a medical emergency that requires prompt and vigorous volume replacement therapy. There are also electrolyte (sodium) and acid-base disturbances. It must be considered that there is a high potential for developing DIC and that stagnant acidaemia blood will promote and/or enhance DIC, which may lead to severe haemorrhage and/or irreversible shock.

**Treatment of Dengue Shock Syndrome**

**Replacement of plasma loss**

Immediate replacement of plasma loss with isotonic salt solution (five per cent dextrose in acetylated Ringer’s solution or five per cent dextrose in normal saline solution) at the rate of 10-20 ml/kg/h, or, in the case of profound shock (Grade IV), as a bolus of ten ml/kg (one to two times), should take place.

In cases of continued or profound shock (with high haematocrit values), colloidal fluid (dextran of medium molecular weight in NSS or plasma) should be given following the initial fluid at a rate of 10-20 ml/kg/h.

Blood transfusion is indicated in cases with profound or persistent shock despite declining haematocrit values after initial fluid replacement.

When improvement is apparent, the rate of IV fluid replacement should be reduced and adjusted one to two hourly throughout the 24-hour period.

Clinical and laboratory signs of Dengue Shock Syndrome which require treatment:

- Cold clammy skin – oliguria,
- tachycardia,
- hypotension or narrowed pulse pressure (less than 20 mm Hg),
- low platelet count (less than 100,000/cu.mm),
- rising Haematocrit values (more than 20 per cent increase).

**Continued replacement of further plasma loss**

Intravenous fluid is continued with the rate adjusted to the rate of plasma loss, as guided by haematocrit values and vital signs, for a period of 24-48 hours (as flow diagram in Figure 2). Establishment of central venous pressure and a urinary catheter may be necessary in the management of severe cases that are not easily reversible.

Colloidal fluid is indicated in cases with massive leakage and to whom a large volume of crystalloid fluid has been given.

In small children, five per cent dextrose in a half-strength normal saline solution (five per cent D/1/2 NSS) is used following initial resuscitation,
Figure 2: Diagram for Treatment of Dengue Shock Syndrome Grade IV

1. Immediate rapid volume replacement with
   - isotonic crystalloid solution
     \[\text{5\% D/NSS}\]
     \[\text{5\% D/RL or Ringer acetated}\]
   10-20 ml/kg/h or as bolus in gr IV

   - improvement
   - no improvement
     \[
     \begin{align*}
     \text{HCT} & \quad \text{HCT} \\
     \text{Blood transfusion} & \quad \text{Colloid} \\
     & \quad \text{Plasma}
     \end{align*}
     \]
     \[
     \text{(Correct acidosis)}
     \]

2. Adjust rate of IV.
   (as in Fig. 2)

and five per cent dextrose in one-third strength normal saline solution (five per cent D/1/3 NSS) may be used in infants under one year of age, if the serum sodium is normal.

Intravenous fluid should be discontinued when the haematocrit reading drops to around 40 per cent and vital signs are stable. A good urine flow indicates sufficient circulating renal volume. A return of appetite and diuresis are signs of recovery. In general, there is no need for fluid therapy for more than 48 hours after onset of leakage and/or shock. Reabsorption of extravasated plasma takes place one to two days thereafter (manifested by a further drop in haematocrit after IV fluid has been stopped and clearing of pleural effusion and ascites has occurred) and may cause hypovolaemia, heart failure and pulmonary oedema if more fluid is given. It is extremely important to emphasize that a drop in haematocrit reading at this stage should not be interpreted as a sign of internal haemorrhage. Strong pulse and blood pressure with wide pulse pressure and diuresis are good vital signs during this reabsorption phase. All of these good signs will help to rule out the likelihood of gastro-intestinal haemorrhage, which is found mostly during the shock stage.

Correction of electrolyte and metabolic disturbances

Hyponatraemia and metabolic acidosis occur commonly in severe cases. Electrolyte levels and blood gases should be determined periodically in severely ill patients and in those with refractory shock. Serum calcium may be low in some cases, particularly in cases with massive plasma and/or blood transfusion. Occasionally hypoglycaemia may develop.

Sedatives

Sedative therapy may be needed in some cases, as when an agitated child needs restraining.
Hepatotoxic drugs should be avoided. Chloral hydrate, orally or rectally, is recommended in a dose of 12.5-50 mg/kg (but not over one g), to be used as a single hypnotic dose.

Oxygen therapy

Oxygen therapy should be given to all patients with shock. However, the oxygen mask or tent increases the apprehension of the patient.

Blood transfusion

Blood transfusion is indicated in cases with significant clinical bleeding, most often with haematemesis and melaena. Fresh whole blood (FWB) is preferable and blood should be given only in volume such that the red blood cell mass becomes normal. Fresh frozen plasma, concentrated platelets and cryoprecipitate are indicated in some cases, when consumptive coagulopathy causes significant bleeding.

Persisting shock with declining haematocrit level (e.g. from 50 per cent to 40 per cent) indicates significant clinical bleeding which requires prompt treatment.

It may be difficult to recognize and estimate the degree of internal blood loss in the presence of haemoconcentration. It is thus recommended to give FWB in small volumes at a time. Insertion of an intragastric tube to determine concealed bleeding or to stop bleeding (by cold lavage) is hazardous and is not recommended. DIC is usually present in severe shock and it may play an important part in the development of massive bleeding and lethal shock. The coagulogram [prothrombin times (PT), PTT and thrombin times (TT)] should be studied in all shock cases to document the onset and severity of DIC which determine the prognosis. Generally anticoagulant therapy is not indicated for DIC. However, in patients with prolonged shock where metabolic acidosis has developed, and in whom shock is refractory to conventional regimens, the use of heparin may be justifiable in order to break a vicious cycle of shock and DIC before the stage of irreversibility is reached. In all instances, extreme caution should be exercised in using heparin.

Blood grouping and matching of every patient with shock should be carried out as a routine precaution.

Essential laboratory tests

In assessing the patient, the following tests are recommended:

- Serum electrolyte and blood gas studies.
- Liver function tests: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum proteins.
- Platelet count, prothrombin time, partial thromboplastin time, and thrombin time.

Evaluation of patient’s condition

Frequent recording of vital signs and haematocrit determinations are important in evaluating the results of treatment. If patients show any signs denoting secondary shock, vigorous anti-shock therapy should be instituted promptly. Patients should be under constant and careful observation until there is a reasonable certainty that danger has passed.

In Practice:

- The Pulse, the blood pressure, the respiratory rate and the temperature should be taken every 30 minutes or more often, until shock is overcome.
- Haematocrit (or haemoglobin) levels should be determined every two hours for the first six hours, then every four hours thereafter, until stable.
- A fluid balance sheet should be kept, recording the type of fluid given, the rate and amount, to evaluate the adequacy of replacement and correction of fluid and electrolytes. The frequency and volume of urine output should be recorded.
During rapid administration of fluid, it is especially important to watch for the following signs of fluid overload/cardiac failure: respiratory distress (with or without crepitation), puffy eyes, sudden increase in the size of the liver, rapid pulse and restlessness.

Management of Patients with Unusual Manifestations

The most importantly encountered are acute hepatic failure and renal failure (which usually follows prolonged shock), which require appropriate treatment. Early exchange transfusion in Reye's syndrome is a life-saving measure, as is haemodialysis in renal failure.

In most cases of shock without severe bleeding, the prognosis is good. The serious pitfall in management of shock is failure to recognize internal bleeding. Over-transfusion with crystalloid and/or plasma fluid instead of blood in these cases is the major contributory factor to the high mortality rate. The major clinical challenge in management of DHF with prolonged shock is often complicated by clinical DIC and massive bleeding. The benefits of anticoagulant and anti-fibrinolysis therapy remain to be further studied. The role of large doses of corticosteroids (pulse therapy) were studied in a small group of patients with prolonged shock and the results were not conclusive. There have been, however, many studies on the role of corticosteroids in the treatment of DSS, which have shown that the therapy is not effective.

With the careful monitoring of patients and appropriate volume replacement as described above, the case-fatality rate of DSS at the Bangkok Children's Hospital is approximately two per cent.

2.8 DHF Special Unit

For the purposes of better care, DHF patients should be put together in a semi-intensive care unit, and for preventive measures this unit must be a mosquito-free area. Paramedical workers or parents can assist in oral fluid therapy and watch for the IV fluid and general status of the patients.

References

Chapter 4
Clinical Laboratory Investigations
by
Pimpan Leangpibul
Prasert Thongcharoen

1. HAEMATOLOGY

Several haematological abnormalities in dengue haemorrhagic fever (DHF) patients have been well described. These can be used as diagnostic aids in proper management and further investigations on the pathogenesis of the disease(1).

1.1 Haematocrit and Haemoglobin

During the first two to three days of the course of disease, the haemoglobin and haematocrit readings are usually normal or slightly decreased. But later on, the first haematological abnormality noted in DHF is the high haematocrit value, which is usually over 40 per cent and may be as high as 50 to 60 per cent(2). Haemoglobin is also much increased, and reaches more than 14 grams per 100 ml. In addition, red blood cell counts of more than five million per mm$^3$ have been observed in about 20 per cent of DHF patients(3). Haemoconcentration is usually observed during the pre-shock and shock phases of the illness and returns to normal during the convalescence stage. The haemoconcentration is due to leakage of plasma into extravascular spaces through damaged capillaries(4). To indicate haemoconcentration, haematocrit determinations should be performed at intervals in patients suspected of DHF in order to give diagnosis and prognosis, leading to proper management. Haemoconcentration is observed in about 20 to 30 per cent of non-shock cases and in about 40 to 50 per cent of shock-death patients (due to severity, haematocrit values cannot be increased). This haemoconcentration also indicates a condition of decreased plasma volume, which is one of the important causes of hypovolaemic shock or circulatory failure(7,9).

1.2 White Blood Cell Count and the Differential Count

The white blood cell count in DHF patients varies from mild leucopenia to moderate leucocytosis. Leucopenia will appear between the first and third day of the disease in 50 per cent of mild cases. During leucopenia there is usually a fairly normal differential count of leucocytes and lymphocytes. The granulocyte concentration is depressed between days three and eight. In severe shock, there is characteristic leucocytosis of $12 \times 10^9/\text{l}$ or more, usually accompanied by an absolute neutropenia. The most striking finding in patients with DHF is the presence of many transformed (or atypical) lymphocytes in peripheral blood smears, usually 20 to 50 per cent, which can easily be demonstrated in buffy coat smear preparations (Figure 1)(10). These transformed lymphocytes can be found in significant numbers (more than ten per cent) as...
early as three days after the onset of fever. This finding may be used as a diagnostic tool to differentiate DHF from other viral as well as bacterial infections, with reliable accuracy, especially in secondary dengue infection. Wells and his associates observed a modest, but progressive post-illness increase of eosinophils in DHF on days 15 and 30 after illness.[11]

1.3 Platelets

Thrombocytopenia is one of the simple diagnostic criteria proposed by WHO for clinical diagnosis of DHF.[12] The platelet count is usually normal during the first three days. Thrombocytopenia in more than 80 per cent of cases begins during the febrile stage and reaches its lowest value during the shock phase of illness (Figure 2).[2,13] The number of platelets rapidly increases in the convalescent stage, and usually returns to normal within seven to ten days after the beginning of the disease. The cause of thrombocytopenia in DHF is controversial. Possibilities include impaired megakaryocyte production or increased platelet destruction. The evidence in this regard is conflicting, since, at the time of the most severe thrombocytopenia, megakaryocyte numbers are often normal or even increased.[14,15] Megakaryocyte dysfunction is observed earlier in the course of the disease.[2] Platelet injury may be due to the virus itself, to platelet-specific antibodies, immune complexes or disseminated intravascular coagulation (DIC).[16] Mitra et al performed isologous platelet kinetic studies in eleven dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) patients, and found that the half-life survival ranged from 6.5 to 53 hours in ten of these patients (normal value: 72-96 hours). Five patients who were studied from 20 days to two years later exhibited normal platelet survival time. Radioisotope study showed that platelet destruction was taking place in the reticuloendothelial system in the spleen and the liver. Besides the quantitative deficit, platelet function was found to be impaired, especially ADP-induced platelet aggregation and ADP-releasing ability.[17,18]

The mechanisms responsible for increased platelet destruction and platelet dysfunction are still unknown. The demonstration of immune complexes (dengue antigen, immunoglobulin and complement globulin) on the surfaces of platelets[19,20] strongly suggests that immune complexes may attack platelets directly and cause their aggregation. The damaged platelets would lose their normal function and then be selectively trapped and sequestered in the liver and spleen. However, it is not known whether dengue antigen-antibody complexes bind directly to the platelets or whether antibody produced against dengue antigen that has already been absorbed on the platelet surface produces platelet aggregation. Either could lead to platelet lysis by complement with the release of vasoactive amines.[21] These immunological mechanisms of platelet damage have already been demonstrated in other viral infections as well as in other conditions.[22-25]

1.4 Coagulation

There has been much speculation concerning the contribution of intravascular clotting to haemorrhage in DHF.[26,27] Tests for clotting function have revealed a prolonged partial thromboplastin time in 54.6 per cent of cases, a prolonged prothrombin time in 33.3 per cent of cases and a normal thrombin time in most cases of DHF.[28,29] When clotting factors were assayed, it was found that several coagulation factors were decreased in variable degrees, including factors II, V, VII, VIII, IX and X (Table). In a WHO collaborative study, platelet count and average minimum fibrinogen levels fell in correlation with the severity of illness, while fibrin degradation products (FDP) rose correspondingly.[30]

Suvatte et al,[29] who studied 149 serologically confirmed DHF/DSS cases in children, observed minimal FDP elevation in DHF patients (11-16 micrograms per 100 ml), but this was not correlated with the severity of the disease. In patients with elevated FDP, there were minimal prolongations of partial thromboplastin and prothrombin times while thrombin times were normal. From the evidence that a slight to moderate increase of FDP was observed intermittently throughout the course of illness in all grades of DHF,[29,30] combined with thrombocytopenia, Suvatte[21] suggested that intravascular clotting was definitely occurring but that DIC was most likely not the major cause of
bleeding. DIC may occur in DHF with prolonged shock and acidosis.

Collaborative studies on haemostasis in DHF patients by Indonesian and Japanese investigators revealed that all DHF patients had manifestations of the acute type of DIC. Prolongations of activated partial thromboplastin time and prothrombin time and decrease of platelet count, fibrinogen, prothrombin, factor VIII, plasminogen and antithrombin III activities were observed transiently during the acute stage of DHF. It was also found that 22 antiplasmin was decreased in the acute stage to 32 per cent of the normal level, on average. This may characterize the haemorrhagic diathesis of DHF patients.

Autopsy investigations of adolescents and young adults, in whom haemorrhage appears to be relatively more severe than in children, have revealed histological evidence for intravascular clotting in small vessels.

The degree of DIC observed in most cases of DHF is not severe. Without anticoagulant therapy, patients usually show rapid recovery after adequate fluid replacement.

1.5 Buffy Coat Smear Preparation

Examination of buffy coat smear preparations is a simple, rapid diagnostic aid used in addition to other clinical manifestations and routine laboratory findings for the correct diagnosis of DHF. Suvarute et al. prepared buffy coat preparations from 120 serologically confirmed DHF patients by carefully cutting the microhaematocrit tubes at the interface of the plasma and red cell column. The thin layer of buffy coat on top of the red cell column was placed on a coverslip to make a smear, which was stained with Wright's stain. Another method of buffy coat preparation, which yields better results, is that of layering about one ml of EDTA blood on top of a methylcellulose-hypaque column in a small test tube to separate the red blood cells. After standing at room temperature for 20 to 30 minutes, the white-cell-rich plasma is removed by
centrifugation in a serofuge for one minute to obtain the white blood cell button. A buffy coat smear is then made from the button in one drop of plasma on a coverslip and stained with Wright's stain. Differential counts are obtained by counting at least 200 cells and calculating the percentage of transformed lymphocytes or "Turk reaction cells". Transformed lymphocytes are defined as large mononuclear cells with fine homologous nuclear chromatin and dark staining cytoplasm, some of which resemble blast cells (Figure 1). The presence of many transformed lymphocytes, usually 20 to 50 per cent, during the first few days of fever is a unique finding in DHF, especially in secondary infections. No such finding has been found during the early phase of illness of other common viral infections such as measles, viral hepatitis and influenza. The buffy coat appearance of DHF is clearly different from that in bacterial infections such as enteric fever, which show many polymorphonuclear leucocytes containing toxic granulations and vacuolizations as well as some monocytes and plasma cells (Figure 2). The early buffy coat diagnosis of DHF correlates well with conventional serological tests. The buffy coat diagnosis of secondary DHF has been shown to be correct in 94.2 per cent of cases as early as the third day of fever.

The reasons for the presence of a high percentage of transformed lymphocytes in DHF patients, especially in secondary infections, are not exactly known. These transformed lymphocytes may represent the anamnestic antibody response to dengue virus since the anti-dengue IgG antibody has been found to rise rapidly to high titres early in the course of secondary dengue infection. If these lymphocytes proliferate and differentiate into antibody-producing cells they are most likely derived from B-lymphocytes. In investigations of subpopulations of lymphocytes in DHF patients, it has been found that the percentage of T-lymphocytes decreases whereas that of B-lymphocytes increases. Thus it is most likely that these transformed lymphocytes are antibody-producing cells. However, the possibility that they may also represent the cell mediated immune response of the host to dengue virus cannot be excluded.
1.6 Bone Marrow

Several groups of investigators have studied the bone marrow of DHF/DSS patients\(^2,8,14,36-38\). Nelson et al showed convincingly that, early in the febrile course of DHF/DSS, the bone marrow is hypocellular with maturation arrest of all elements. The maturation arrest of megakaryocytes was more pronounced than that of other elements. There was a definite increase in number of small lymphocytes, monocytes, reticulum cells and phagocytic clasmatocytes. From the fifth to the eighth day of illness the bone marrow showed a sudden accelerated erythropoiesis and many young megakaryocytes. In the convalescent stage, the bone marrow cellularity moderately increased, while erythroid hyperplasia occurred and megakaryocytes of both mature forms moderately increased in number. All patients showed very active platelet production.

2. BLOOD CHEMISTRY

The number or degree of laboratory abnormalities generally increases in correlation with the severity of illness.

2.1 Serum Protein

There is pronounced hypoalbuminaemia in shock cases of DHF patients with the total serum protein lower than in non-shock cases. The proportion of the various serum proteins, on electrophoresis, is within normal limits\(^39\).

Serum protein of lower than 4.5/dl has been observed in 78.5 per cent and 31 per cent of shock and non-shock cases of DHF respectively\(^37\). The study of Phongphanich et al\(^3\) showed serum albumin to be 2.55±0.46 g/dl in severe cases and total protein to be 5.5±0.87 g/dl.

2.2 Liver Function

Serum alanine aminotransferase (ALT or SGOT) and serum aspartate aminotransferase (AST or SGPT) are slightly elevated in severe cases, but there is no statistically significant difference between shock and non-shock cases\(^3,40\). Sero-flocculation tests (thymol turbidity and Kunkel) are normal. The mean serum bilirubin concentration (total, conjugated and unconjugated) is also within normal limits and there is no increase of urinary urobilinogen\(^39,41\). Serum alkaline phosphatase and serum gamma-glutamyl transferase (yGT) activities are within normal ranges\(^39\).

The fact that SGOT activities are elevated in severe cases seems to be coincidental with the pathological finding that liver cells are acutely damaged. The increased level of these enzymes is presumably due to the release of enzymes from damaged cells caused by disturbed cellular permeability. In addition, the possibility that the SGOT/SGPT elevation might be observed in other conditions such as anoxia, which also happens in severe DHF with shock, should be considered. The normal serum alkaline phosphatase and serum gamma-glutamyl transferase activities suggest that biliary tract excretion is not damaged. The normal serum bilirubin concentration suggests that there is no sign of hepatotoxicity in patients with DHF.

2.3 Electrolytes, Water Balance and Blood Gas

Daily measurements of serum electrolytes in DHF patients show definite decreases in sodium and chloride levels, especially in shock cases (average 125 mmol/l of Na\(^+\) and 91 mmol/l of Cl\(^-\)), which return to normal in the convalescent stage\(^42,43\). The level of serum potassium is in the range of high to normal. Water balance studies have indicated that hyponatraemia is the result of salt depletion in excess water available from increased metabolism and, possibly, decreased renal excretion in shock cases\(^43\).

Furthermore, in hyponatraemic patients, hypo-osmolality of urine briefly accompanies plasma hypo-osmolality, raising the question of a transient period of inappropriate anti-diuretic hormone (ADH) excretion. Findings have included low blood CO\(_2\), somewhat reduced serum base excess and a somewhat elevated serum pH, consistent with
alkalosis and mild metabolic acidosis in all cases\textsuperscript{42,43}.

2.4 Serum Acid Phosphatase

Studies conducted by Burke \textit{et al.}\textsuperscript{44} and Lam \textit{et al.}\textsuperscript{45} showed serum acid phosphatase to be at higher than normal levels in children affected by DHF. During the acute phase of illness the serum acid phosphatase activity was 63.7 U/l higher than that of normal children (average normal value = 31.5 U/l)\textsuperscript{45}.

3. CEREBROSPINAL FLUID

The cerebrospinal fluid (CSF) is usually normal in colour and pressure, except with occasional blood stains. Lymphocytes, from five to ten cells per mm\(^3\), may be present. The Pandy test may be weakly positive, with the total protein between 50-100 mg\textsuperscript{41}.

4. RENAL FUNCTION, URINE AND STOOL EXAMINATION

Urine is usually normal but occasionally one may encounter a trace to one plus of albumin. Red blood cells are seldom seen unless the urinary tract has been complicated with haemorrhage. In one study, thirty-six per cent of patients showed transient proteinuria (1 + to 3 +) with occasional microscopic haematuria. Mild elevation of serum urea nitrogen and serum creatinine was also observed\textsuperscript{46}.

Stool may not give a positive benzidine test unless there is gastro-intestinal tract bleeding. The chance of getting a positive reaction is greater if this test is done during the toxic phase of the disease.

5. IMMUNOCHEMICAL STUDIES

5.1 Serum Complement

Serial studies of serum complement C3 levels revealed that the C3 level definitely decreased to 34 to 45 per cent of normal values during the shock phase of illness (day four to day six), in proportion to the severity of the disease (Figure 4)\textsuperscript{29,47}. In each grade of DHF, the depression of C3 level coincided with the reduction of platelet count, and the C3 immunofluorescent staining of platelets was positive in 13 of 18 patients, with homogeneous patterns\textsuperscript{19}. When all types of complement were measured, it was found that complement depression in DHF involved primarily C3, C3 proactivator (C3PA), C4 and C5, with marked depressions in shock cases (Figure 5)\textsuperscript{48}. The high correlation observed between the lowest C3 values and the lowest C3PA values suggest that C3 is consumed through the activation of both the classical and alternative pathways. However, extravasation of complement into extravascular space or decreased synthesis can certainly not be excluded as causes of complement reduction. A study of C3 levels in pleural fluid\textsuperscript{41} and radioisotope studies of complement metabolism\textsuperscript{49} support the hypothesis of complement activation rather than decreased synthesis or extravasation of the complement.

![Figure 4. Serial determination of serum complement C3 in dengue haemorrhagic fever patients plotted against day of the disease.](image-url)
5.2 Kinin System

A study conducted by Edelman and his associates indicated that there was significant evidence for activation of the key substrates in the serum kinin system, but the authors believed that the kinin system was not the mediator responsible for increased vascular permeability.

5.3 Histamine

Studies of 24-hour urinary histamine in DHF patients revealed significantly increased urinary excretion of both free and total forms. The finding suggests that histamine may be one of the mediators, if not the only one, released during the course of the disease, especially in severe cases. Histamine may play an important role in the leakage of intravascular fluid into the various serous spaces, resulting in hypovolaemia and shock.

6. HORMONAL STUDY

Serial studies of serum cortisol in DHF patients revealed that the cortisol level was definitely elevated to between four and eight times the normal level during the shock phase of illness, in proportion to the severity of the disease. Sequential determinations of cortisol demonstrated a relatively low level in grade IV as compared to grade III, which might be due to the leakage of cortisol into interstitial spaces and serous cavities or to adrenal suppression, either by exogenous steroid therapy or by adrenal exhaustion in some fatal cases.

7. ELECTROCARDIOGRAPHY

In 1967, Wong et al. reported electrocardiographic abnormalities in 44 per cent of children with DHF. The possibility of myocardial involvement as the cause of shock in DHF was postulated. Since treatment is different for cardiogenic and
Figure 6a. ECG of a dengue haemorrhagic fever patient, recorded on admission and in shock stage.

Figure 6b. ECG of same patient (as in Figure 6a) recorded one week later (completely recovered).
hypovolaemic shock, Phongphanich et al evaluated cardiac function in 50 children with serologically proven DHF[44]. Sixty-four per cent of them showed electrocardiographic abnormalities (Figure 6a and 6b). The most common findings were ST-T wave changes and there was good correlation between severity of the disease and the electrocardiographic abnormalities. The finding that patients with electrocardiographic evidence of myocardial involvement have a normal central venous pressure lead to the assumption that there is no central circulatory failure in DHF. Most likely, cardiac impairment is a consequence of prolonged shock, secondary to hypovolaemia.

8. RADIOGRAPHIC STUDY

Chest roentgenograms are usually normal[44]. However, at least one-half of children have pleural effusions, which can be demonstrated on X-ray films[44]. The incidence of pleural effusion may be encountered more than has been reported, depending on the time of the roentgenologic study.

References

Clinical Laboratory Investigations


Chapter 5
Pathology of Dengue Haemorrhagic Fever
by
Natth Bhamarapravati

1. INTRODUCTION
DENGUE haemorrhagic fever (DHF) broke out on the scene of South-East Asia in 1957. A thorough search of autopsy records of cases performed before World War II, in the file of the Department of Pathology at the Faculty of Medicine, Siriraj Hospital in Bangkok, Thailand, which include several hundred case of well documented autopsy, has revealed no cases which can be construed as DHF clinically or pathologically, indicating that the disease most likely did not occur in epidemic or sporadic form during those days in Thailand. Inquiries of this nature at other departments of pathology in South-East Asian countries have also failed to yield information on the possibility of DHF occurring before the second world war.

Autopsy study of DHF cases in the 1960s appears to be inconclusive. Virological diagnosis of these cases was difficult because the virus disappears from the blood in most cases after the end of the febrile period, when shock begins. However, in several studies a number of dengue viruses of all four types were recovered from organs of patients who died from DHF. Haemagglutination Inhibition (HI), Complement Fixation (CF) and the Neutralization Test (NT) were the main diagnostic tools in the 1960s, but required weeks of work and were in need of refinement. A judicious review of clinical records, together with good autopsy data showing some well recognized pathologic features, could yield a fairly accurate diagnosis, especially when the patients came from urban settings of South-East Asia during the epidemic rainy seasons. No other febrile diseases in this part of the world are known to cause death from hypovolaemic shock.

The pathology of DHF is not pathognomonic. Certain variables may have to be considered when, with very few exceptions, pathologic findings are based on material obtained at autopsy, such as those cases which have been in the febrile stage for a few days and then go through the shock stage, and who usually die within 24 hours of shock. The pathologic manifestations of organ systems may be complicated by those associated with shock, anoxaemia and related conditions, which may make the primary pathologic features more complicated to recognize. The pathological changes may vary among different age groups. It is apparent from epidemiological studies as well as sporadic reports that cases of dengue haemorrhagic fever may occur in:

(a) Infants less than one year old with primary dengue infection,
(b) Children of preschool and school age with a second attack of dengue infection (the greatest number of DHF cases are in this category),

(c) Adolescents or young adults with a second attack of dengue infection, and

(d) Children or adults with primary dengue infection (this type of case is rather infrequent but carefully studied cases have been documented).

The pathologic responses in subjects belonging to categories (a) and (b) have been extensively studied and reported. A few cases in category (c) have also been documented, but autopsy studies of cases among category (d) have not been reported.

2. PATHOLOGY IN FATAL CASES

Fatality in DHF usually occurs within 24 hours after the onset of shock. For those who survive, recovery is usually rapid and there are no well recognized sequelae of the disease. This corroborates findings at autopsy which have suggested that death, in most instances, is due to failure of some key homeostatic mechanisms. If recognized and counteracted in time, irreversible cellular and organ pathology can be precluded. Even though there is no real pathognomonic lesion in dengue haemorrhagic fever, at the autopsy table, when a large number of cases are reviewed, certain patterns emerge. Most of the subjects appear well developed and well nourished. Gross findings may reveal some degree of haemorrhage in the skin and subcutaneous tissue. Rare cases may show evidence of icterus and would be difficult to differentiate from hepatitis, but confirmatory virological studies have been made. Haemorrhage may appear as haemorrhagic rash, petechial or ecchymosis, especially around needle puncture marks. The rash may be particularly striking over the lower limbs. Frank haemorrhage may appear in patches in the subcutaneous tissue. The heart typically shows flame shaped subendocardial haemorrhage in the left ventricular septum. Occasionally subendocardial haemorrhage over the papillary muscles is observed.

Many hearts, on autopsy, show an acute angle of the apex of the left ventricular septum, and the myocardium of the left ventricular chamber appears thick and firm to the touch, indicating that the heart has stopped in systole. This could also be considered a major finding in hypovolaemia, when the left ventricular chamber is not filled to its full volume. It is possible that some electrolyte imbalance or abnormality in the functioning of the conduction pathway may lead to stoppage of the heart in systole. Haemorrhage may also be present in the mucoea of the nose, gum and gastro-intestinal tract, as well as underneath the capsule of the liver. While haemorrhage may be striking, especially in cases of prolonged shock in particular among adolescents and adults, the amount of haemorrhage is not excessive and frank bleeding into the serous cavities is rare. In only two cases has the author found gross haemorrhage into the adrenal cortical tissue of the type seen in Water House Friederichsen syndrome. The meninges and brain may show, at most, petechial haemorrhage. Massive haemorrhage into the brain and spinal tissue have not been reported. Serous effusion with a rather high (more than 1 g/dl) protein content is commonly present in the pleural and abdominal cavities, and occasionally in the pericardial space. The amount of effusion, when considered together with the fluid intake and fluid loss in the 24 hour period before death, shows that there is a significant amount of fluid loss into the extravascular compartment. Analysis of the effusion shows a high protein content, mostly of albumin and small molecular weight globulin, thus rendering the nature of effusion exudative rather than transudative, even though there is only a small number of leucocytes in the fluid. Analysis of the plasma in the heart blood reveals correspondingly low levels of protein, especially of albumin. The most striking findings at autopsy are the negative findings. There is no major organ damage at the gross pathologic level, and neither is there any evidence of secondary infection such as bacterial pneumonia, cystitis or pyelonephritis.

At the light microscope level, medium and small blood vessels do not show significant alteration in the vascular wall. Occasionally capillaries and venules in affected organ systems may show perivas-
cular haemorrhage and perivascular infiltration by lymphocytes and mononuclear cells. In the lung, kidney, liver and splenic vasculatures, an increase in the number of young megakaryocytes is observed. In adolescents and young adults, where haemorrhage appears to be relatively more severe than in children, histological evidence of intravascular clotting in small vessels has been recognized. In the lymphoid tissue, especially of subjects in categories (b) and (c), there is a marked increase in the activity of the B lymphocyte system, with active proliferation of plasma cells and lymphocytoid cells, and very active germinal centres. There is a marked proliferation of large immunoblastoid cells and a considerable turnover of lymphocytes manifested by a reduction of the white of splenic pulps, lymphocytolysis and marked lymphocytic phagocytosis. Some investigators have alluded to a decreasing number of lymphoid cells in the T lymphocyte - dependent area of lymphoid organs.

The liver may show changes which can be considered as close to pathognomonic as can be, even though similar findings have been recognized in the livers of other viral haemorrhagic fever cases such as yellow fever, Lassa fever, Ebola fever and Marburg disease. There is focal and coagulation necrosis of liver cells. Small foci of necrotic liver cells may be seen in the paracentrolobular or mid-zonal regions. Swelling of Kupffer's cells with hyaline necrosis, and formation of acidophilic cells with fat vacuoles, the so-called "Councilman" bodies, are observed. The liver cells may show some mild degree of fatty metamorphosis. Mononuclear leucocytes and rare polymorphonuclear leucocytes are occasionally seen in the sinusoids, and at times in the portal areas. The lesions in the liver resemble those in experimental yellow fever in monkeys, with spotty focal necrosis, 72-96 hours after the virus inoculation, when there is no extensive parenchymal cell damage. In rare fatal cases, the liver changes are rather extensive and clinical jaundice has been recognized.

The lungs show thickening of interstitial septa, with increases in the numbers of mononuclear cells, alveolar macrophages and megakaryocytes. These changes have been considered by some as interstitial pneumonia, but such pneumonia has not been substantiated by clinical and X-ray studies. The brain and spinal cord show evidence of perivascular oedema and haemorrhage but no evidence of meningitis or encephalitis. The nerve cells show some increase in acidophilia but no neuronal necrosis and no glial proliferation are seen.

Kidneys have revealed increases in cellularity of the glomerular capillary tufts and some nonspecific, or anoxic, tubular changes. It is interesting to note that, even though autopsied children may have been in shock for some period of time before death, no evidence of acute tubular necrosis or bilateral cortical necrosis has been observed.

The adrenal glands show depletion of lipid in the adrenal cortex. Congestion and focal haemorrhage may be noted in some cases.

Myocardial fibres show non-specific anoxic changes and there is no evidence of myocarditis except oedema and focal perivascular haemorrhage. Rare reports of "myocarditis" may be found in the literature.

Gastro-intestinal tracts are oedematous and show haemorrhage into mucosa, submucosa, muscularis and also serosa. Marked congestion and haemorrhage are present in cases with prolonged shock.

In summary, the pathology of DHF in fatal cases generally reveals no gross or microscopic evidence of severe organ pathology that could explain the cause or causes of death. Those patients who were in prolonged and profound stages of shock before death, especially young adults, may show consequences of shocked organs, intravascular clotting and more haemorrhage than others. There is no evidence of primary or secondary infection in the lungs or other organs, which might imply an immunocompromising situation during the terminal stages. Despite some display of cerebral symptoms, i.e. "encephalopathy", there is no convincing evidence of "encephalitis", i.e. neuronal damage due to dengue virus.

Pathological studies have also been conducted on tissues obtained from living patients by biopsy. These tissues include bone marrow, kidney, and
Depression of bone marrow has been observed in the febrile period. The depression involves most blood elements. At the time of shock, and as revealed in most fatal cases, the bone marrow may appear normocellular or even hypercellular. Young megakaryocytes seem to proliferate and enter the circulation in high numbers. They are seen to lodge in the blood vessels of visceral organs, such as lungs, liver, kidney and spleen, and are seen on autopsy. The kidneys show immune complex glomerulonephritis with deposition of immunoglobulin and complement on the glomerular capillaries. There is little, if any, fibrinogen or fibrin deposit. Dengue antigen has not been found in the glomeruli. Mild to moderate haematuria and proteinuria are clinically recognized in some subjects, and disappear in about three weeks, indicating that the conditions represent a transient immune complex glomerulonephritis similar to that occurring in some infectious diseases. No kidney disease sequelae are ever recognized.

Skin rashes in DHF have been described in several forms such as petechial, maculopapular, or measles-like rashes, and the exposed lower limbs are frequently involved. Biopsy studies show that the microvasculature located in the dermal papillae is the main site of injury. The basic changes include swelling of the endothelial cells and perivascular tissues. Some erythrocytes can be seen outside the walls of the vessels. There is increasing infiltration of cells around the perimicrovasculature tissue by mononuclear phagocytes and lymphocytes and other unidentified cells. Immunoglobulin, mostly IgM, complement and fibrinogen are located on the vessel walls. More fibrinogen is detected in cases with haemorrhagic rash. Dengue antigen can also be demonstrated in the cells surrounding the microvasculature, mostly in mononuclear phagocytes. In some skin biopsies, cell infiltration may spread out underneath the epidermal-dermal junction, especially in those with maculopapular types of reaction. While the pathologic features of these rashes are suggestive of antibody dependent Arthus types of reaction, there is no evidence of necrosis of the vessel wall or of vasculitis. No significant infiltration of polymorphonuclear leucocytes has been observed in the lesions. The site of the immunopathologic lesion in DHF, centring around the microvasculature in dermal papillae, is different from that of immune complex mediated, systemic lupus erythematosus where the immune complex is usually deposited in bands along the epidermal-dermal junction. Clinically, most surviving DHF patients show full recovery from their skin changes in a few weeks, even though some vasculopathy may persist and positive tourniquet tests, in some cases, are reported up to three weeks after recovery from shock. Electron microscopy of the microvasculature of the skin shows non-specific changes but indicates increasing transport activities of the endothelial cells. Dengue virus has not been identified in any cells in electron microscopic studies.

3. LOCALIZATION OF DENGUE ANTIGEN IN HUMAN TISSUE AND POSSIBLE SITES OF DENGUE VIRUS REPLICATION IN THE BODY

Attempts have been made to locate dengue antigen in the tissues of fatal cases and in biopsy material by using immunofluorescence, both direct and indirect methods, and the immunoperoxidase method. In autopsy material, dengue viral antigen is localized in cells of reticuloendothelial origin, such as those cells lining the Biotroh cords in the spleen, cells in the cortex of the thymus, Kupffer cells and flat sinusoidal lining cells of the liver, alveolar macrophages, and possible mononuclear phagocytes in the skin (no attempt to differentiate Langerhans' cells in the skin has been made). In cases belonging to category (a) (infants less than one year old with primary dengue infection), cells containing dengue antigen are found in relatively large numbers, while in subjects belonging to categories (b) and (c) (children or young adults with a second attack of dengue infection) relatively fewer dengue antigen containing cells are detected in the various organs. Circulating monocytes have also been shown, by both immunofluorescence and electron microscopy, to carry dengue antigen.
Such cells can also support replication of dengue virus. A small percentage of B lymphocytes have been shown to present dengue antigen on the surface, especially during shock. The B lymphocyte continuous cell line (Raji cell line) can support dengue virus replication in the cytoplasm and expresses the virus on the surface. T lymphocytes have yet to be shown to support dengue virus replication. Human blood platelets have been demonstrated to show the presence of dengue antigen, as well as immunoglobulin and complement, on the surface membranes, suggesting that peripheral destruction of platelets may also occur through immune complex mechanisms. Rabbit megakaryocytes have been shown to support dengue virus replication in vitro studies. This could lead to an increase in destruction of megakaryocytes, or to maturation arrest and early release (young megakaryocytes are seen in increasing numbers in the small vessels or visceral organs), or to shedding of morphologically or functionally abnormal platelets during infection. Giant platelets and a short life span of platelets have been demonstrated. A combination of these possible abnormalities can only be speculated upon.

### 4. PATHOPHYSIOLOGY AND PATHOGENESIS

The large quantity of serous effusion with high protein content, and the lack of major organ injury, as well as some of the clinical studies using general laboratory and dilution techniques, suggest that the general vascular system is the major site of injury, with two major effector pathways of expression: one, leading to a leakage of erythrocytes; and two, leading to a leakage of water, electrolytes and plasma protein, and resulting in hypovolaemic shock. Since erythrocytes are larger than albumin molecules, the pores that each passes through may be different and separate, yet related mechanisms may be in operation. Because the leakage of water, plasma protein and electrolytes occurs rather rapidly and recovery also occurs within 24-48 hours in surviving cases, without apparent severe morphological changes, mediators of some kind such as C3a, C5a, histamine, bradykinin and others are implicated. The mechanism of haemorrhage is a complex one and may result from a combination of factors, such as thrombocytopenia, coagulation defects, and vasculopathy as a result of interaction between platelets, complement activation products, plasminogen and fibrin activated products. Laboratory studies have shown a prolonged prothrombin time with low levels of coagulation factors II, V, VII, IX and XII. Fibrinogen levels are lowered and have been shown to be related to an increase in consumption of fibrinogen. Small amounts of fibrinogen split products have been detected, but these are not at the level commonly encountered in classical disseminated intravascular clotting. The amount of haemorrhage is not excessive and a chromium 51 label study did not reveal a lower red blood cell volume. This situation may be different in adolescents and young adults. Special studies have demonstrated that blood levels of complement components Clq, C5, C5-8 and C3 proactivator decrease and that C3 catabolic rates increase. These findings suggest a complement consumption process via the classical pathway and perhaps via the alternate pathway as well. Severe complement activation has also been demonstrated in cases of DHF related to primary dengue infection. In a series of in vitro studies, using the amount of complement split products as determined by immunoelectrophoresis, dengue viral antigen from several sources such as mouse brain antigen, LLC-MK2 cultured cells, and infected human monocyte culture, formed dengue antigen antibody complexes which could activate complement C3. Complement split products have also been detected by this method in patients mostly in grades III and IV. Other in vitro studies have shown that dengue virus can bind with platelets directly or with antibody, which results in platelet destruction. It has also been suggested, on theoretical grounds, that initial bleeding may be aggravated after complement activation, which may yield C3a and C5a and other vascular permeability factors and lead to the formation of bradykinin after activation of factor XII. The presence of...
thrombin and bradykinin may release plasminogen activator and FG12 from the vascular endothelial cells. Also, aggravated bleeding may be postulated through the involvement of protein C by thrombin-thrombomodulin complexes. Activated protein C degrades factors Ca and VIIa and increases the plasminogen to plasmin conversion by decreasing antiplasminogen activator. Based on this speculation, the complement, coagulation, plasmin, kinin and arachidonic acid cascade systems are all implicated. Some of the systems act to increase vascular permeability but others act on leakage of erythrocytes. Only some of these systems have been studied in actual clinical situations in DHF subjects, with rather confusing results.

The pathogenesis of dengue haemorrhagic fever may be considered to result from direct cellular injury by dengue viruses or from immunologically mediated injury. A combination of both mechanisms has been demonstrated in endothelial cells in in vitro studies, but so far no evidence that the virus can grow in human endothelial cells in an in vivo situation has been demonstrated. The fact that all four types of dengue virus have been isolated from fatal cases seems to favour direct cell injury by the virus. Other observations are, however, not favourable to direct cellular pathology by the virus. These include the following facts: that a second infection is a risk factor; that no animal model of DHF has been discovered, despite the fact that practically all other viral haemorrhagic fevers have counterpart animal models, including yellow fever viral infection which is very closely related to dengue and is in the flaviridae family; that major organ pathology is not evident at the time of death; that for those DHF patients who survive the crisis there are no sequelae; that vascular changes are reported to be non-specific from the morphological standpoint at the light and electron microscope levels; and that it is difficult to locate dengue virus antigen in cells other than reticuloendothelial cells. Immune complex mediated injury has been considered, but again it is difficult to view this factor in perspective. Some findings, such as disseminated intravascular clotting, seem to be secondary to the main pathologic events. Recent work on locating dengue antigen in cells of the reticuloendothelial system, such as the mononuclear phagocyte series of both fixed and circulating kinds, and the knowledge that at least in circulating mononuclear phagocytes dengue replication can take place, suggest that monocytes may be important in the pathogenesis of this disease. The entry of the virus into such cells is promoted by immune complexes formed between anti-dengue antibody and dengue virus. The anti-dengue antibody, when circulating at non-neutralizing levels or when directed at the viral epitopes which do not result in neutralization, is termed infection enhancing. Activated monocytes in the circulation have been found, in several unrelated experiments, to release substance(s) that can activate the clotting system and the complement system and that also modify vascular permeability. This has lead to the notion that monocyte-dengue virus interaction is important in DHF. The more monocytes are infected by dengue virus, as in a secondary infection, the more severe is the disease. This may be the reason why there are fewer fixed mononuclear phagocytes containing dengue antigen (i.e. supporting dengue virus replication) in subjects with secondary dengue infection. The main cells supporting dengue replication in this instance are probably the circulating ones conditioned by enhancing anti-dengue antibody rather than fixed mononuclear phagocytes. It has also been shown that several types of product, which include bacteria related products and even endotoxin, can enhance the monocyte's propensity for increasing dengue replication. This offers the possibility that other risk factors for increasing dengue virus replication in vivo may be operational in DHF patients.

The lack of an animal model makes it imperative that carefully conducted detailed clinical studies are carried out to clarify many of the unresolved problems. Yet the relatively rapid clinical changes, and the short period of time during which mediators are released, makes clinical studies very difficult, especially when any invasive measures to extract tissue and cells may compromise the host by bleeding.
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Chapter 6

Pathophysiology and Pathogenesis of Dengue Haemorrhagic Fever

by

Scott B. Halstead

1. PATHOPHYSIOLOGY

1.1 Water and Electrolyte Shift

As demonstrated with the dilution technique using $^{131}$I human albumin$^{(1)}$, dengue haemorrhagic fever (DHF) is characterized by the shift of fluid and protein, predominantly albumin, out of the vascular compartment into interstitial spaces and serosal cavities. This results in the decreased plasma volume which is observed in dengue-infected children three to seven days after onset of illness or about the time of defervescence. Plasma volume correlates inversely with the severity of disease. Prior to treatment, as demonstrated by the use of $^{82}$Br and $^3$H$\text{H}_2\text{O}$ studies$^{(2)}$, there is no change in total body water, nor is there a shift of water into or out of cells. Later, with parenteral fluid therapy, total body water increases as evidenced by increase in body weight and oedema$^{(2)}$. Since the red blood cell volume does not change$^{(1)}$, the venous haematocrit reading can be used as a reliable measure of plasma volume loss.

The rapid and life threatening decrease in blood volume is mediated by endothelial defect(s) which have not been fully characterized. It seems evident that vascular “pores” are created which admit smaller proteins such as albumin but exclude globulins. Serosal fluids may show an albumin concentration between 80 per cent and more than 100 per cent that of plasma$^{(1)}$. The character of the transudate in DHF has been studied by Chavalittamrong et al.$^{(3)}$, who analysed pleural effusion composition in 29 DHF patients. The average total serum protein at the time of study was 5.9 g/dl. The albumin concentration in pleural fluid was 83.4 per cent of that in serum, while IgG was 56.4 per cent. Smaller molecular weight solutes such as uric acid, creatinine and urea equilibrated in both fluids. Reversals in the A/G ratio are common in DHF, especially in patients with hypotension$^{(4)}$. It is unlikely that these losses occur through the urinary or gastro-intestinal routes since proteinuria is both transient and insignificant$^{(5)}$ and there is no evidence clinically or at autopsy of fluid pooling in the bowel$^{(6)}$.

Although by light microscopy there are no changes in endothelial cells or loss of integrity of capillaries$^{(6)}$, by electron microscopy endothelium shows changes consistent with cellular injury due
to anoxia or burn(7). Such studies were done on skin biopsies from 44 DHF patients taken during the acute illness phase from sites of purpura, petechiae or maculopapular eruption. Observations included swelling of endothelial cells, dilatation of the rough endoplasmic reticulum and nuclear envelopes, swelling of mitochondria and, in some areas, thinning of endothelial cells. These findings are consistent with those observed in animals given histamine or serotonin or made thrombocytopenic(9). In some sections enlarged endothelial gaps were seen. Necrosis of endothelial cells was not observed. These findings are consistent with the possibility that vascular permeability is pharmacologically mediated as the result of loss of tight cell-to-cell junctions.

The acute stage of DHF is characterized by anoxaemia, hyponatremia, low blood PCO₂ somewhat reduced serum base excess and a somewhat elevated serum pH. These findings are consistent with a respiratory alkalosis combined with a mild metabolic acidosis(2). Respiratory rates are elevated in DHF in direct proportion to the severity of disease(8). Additional factors contributing to respiratory alkalosis may be fever and salicylate therapy. It has been suggested that hyponatremia in DHF may be caused by a combination of reduced salt intake secondary to anorexia, sweating and vomiting plus an increase in plasma water, possibly the result of decreased renal perfusion plus the increased metabolic production of water due to fever(2). Apparently, hyponatremia is not due to release of anti-diuretic hormone (ADH) since there is not the expected hypernatraemia or negative sodium balance(2).

1.2 Blood Pressure

Studies on dengue-infected children consistently demonstrate reduced central venous pressure, indicating that congestive heart failure is not involved as a mechanism of shock(6). Although low voltage, elevated ST segments and flattened T waves occur frequently during shock, the consensus is that these are probably due to anoxaemia and decreased cardiac persuasion rather than to direct myocardial damage(16-13). There is increased peripheral vascular resistance, decreased cardiac output consistent with venous pooling, plus loss of fluid by transudation(10). It has been suggested by Futrakul et al(14) that low creatinine clearance in the presence of low central venous pressure is consistent with a constriction located in post-capillary arterioles. Whether this is due to stimulation of adrenergic receptors is not entirely clear(14). Two adrenergic blocking agents, debenzaline and effortil, when infused in DHF children with low central venous pressure, regularly increased creatinine clearance but did not change central venous pressure.

Shock is not caused by reduced plasma renin, since plasma renin levels studied in 24 DHF cases were actually increased in proportion to disease severity(15). Mean values on days four and five in shock cases were nearly twice as high as values for normal children, and were considerably greater than can be accounted for by haemoconcentration.

Of uncertain significance are the observations by Parshad et al(16) of decreases in T₄, T₃, free thyroxine index, and thyroid-stimulating hormone (TSH), and increases in T₃ uptake in 15 Caribbean adults with acute dengue fever.

In contrast to the reduction of plasma volume during shock, there is in the later stages of the disease a marked increase in central venous pressure, hypervolaemia and congestive heart failure(1,9,12,17,18). During this period great care must be taken not to administer parenteral fluid in order to avoid causing death. Similarly, transfusion of whole blood should be avoided in children who have markedly elevated haematocrit readings.

Finally, three dengue shock syndrome (DSS) cases studied at or near death had markedly elevated serum potassium levels(4). A terminal electrocardiogram was consistent with hyperkalaemia. Despite this evidence for a mechanism in the fatal termination of shock, the use of ion exchange resin, peritoneal or renal dialysis has not been reported.

1.3 Platelets

The second consistent attribute of DHF is thrombocytopenia. In fact, the presence of a reliable
platelet count of 100,000/mm² in an individual with a confirmed dengue infection together with objective evidence of increased capillary permeability, usually an elevated haematocrit reading, plus low serum protein⁹⁻¹⁰, is essential for case definition. Platelet numbers fall during the febrile phase of dengue infection, in some instances two or more days prior to onset of hypovolaemia. Thrombocytopenia is also seen in children who do not have an elevated haematocrit. Thus thrombocytopenia is the most sensitive screening test for DHF although its specificity is not known. The number of platelets increases rapidly during convalescence, rebounding to 25-50 per cent in excess of normal. In a single case, the platelet rebound following dengue 1 infection was observed to be accompanied by high serum levels of thrombopoietic stimulatory activity²¹.

Studies in DHF patients have suggested two mechanisms for thrombocytopenia: depression of megakaryocyte function²²⁻²⁵ and increased destruction of mature platelets²⁶. Bierman and Nelson²⁷ obtained serial bone marrow biopsies from a young adult Caucasian Peace Corps volunteer with dengue fever. Bone marrow taken on day four after onset of fever was hypocellular. Megakaryocytes were reduced in number; there was diminished erythropoiesis and totally absent granulocytopenia. On successive biopsies three and six days later there was a progressive return to normal cellularity. A similar bone marrow picture has been described in DHF patients studied early²²⁻²⁴, although during shock and at death bone marrows have often been normocellular or hypercellular²²⁻²⁹. Mittrakul et al²⁶ performed isologous platelet kinetic studies in DHF/DSS patients and found half-life survivals which ranged from 6.5 to 53 hours in ten patients (normals: 72-96 hours). Five patients were studied 20 days to two years later and exhibited normal platelet survival times. Radio-labelled platelets showed increased localization to liver rather than to spleen. In convalescence, the spleen: liver platelet localization ratios returned to normal. Platelet function studies were done on 13 patients, but not until days nine to twelve after the onset of fever, when platelet counts were normal. In these patients, release of platelet adhesiveness factor (PF3), aggregation in the presence of DP, collagen and thrombin were normal. Clot retraction time was normal. Of interest was the fact that none of the platelets released ADP. There is one report in the literature of increased platelet adhesiveness in Vietnamese children hospitalized with a diagnosis of DHF²⁰. In 37 cases platelet adhesiveness was reduced and in the remaining 14 it was increased. It should be noted that all patients studied had platelet counts of greater than 100,000/mm², raising serious questions about the identity of the syndrome studied³⁰.

The mechanism of increased destruction of platelets during dengue infection is not known. Basanta Otero et al³¹ studied 32 adults with serological evidence of a secondary dengue infection (dengue type 2), all with thrombocytopenia and none of whom were found to have had platelet anti-antibodies using the platelet suspension immunofluorescence test. Thrombocytopenia occurs regularly in infants with DHF admitted to Bangkok Hospitals³²⁻³⁵. Nearly all of these infants experience a primary dengue infection³³. This observation makes it unlikely that antigen-antibody complexes are responsible for platelet destruction. However, these patients have the same frequency and magnitude of complement activation as do the children experiencing secondary dengue infection³²⁻³⁴. Thus, active complement components, dengue virus itself, damaged endothelial cells and activation of the blood clotting system remain as possible mechanisms of thrombocytopenia separately or in combination.

1.4 Coagulation factors

DHF is characterized by prolonged bleeding times, normal venous clotting time, prolonged silicone clotting time²⁴⁻²⁹, moderate prolongation of the one stage prothrombin time, prolongation of the activated partial thromboplastin time, decreased serum fibrinogen levels and in severe cases, increased fibrinogen degradation products (FDP)³²⁻³⁴⁻⁻³⁸. In DHF patients with moderate
prolongation of the prothrombin time (PT), a decrease has been observed in factors II, V, VII and X in patients whose enzyme levels have suggested liver damage. In eleven Indonesian children with DHF (three DSS cases), decreases in factor VII and antithrombin III activities were noted. There was a remarkable decrease in alpha 2 antiprotease with a concomitant decrease in plasminogen. In the WHO collaborative study, platelet counts and average minimum fibrinogen levels fell in correlation with the severity of illness, while FDP rose correspondingly. Bokish et al. observed a mild increase in FDP in DHF/DSS, but were unable to show a direct correlation with severity of clinical illness. In the study by Srirkaikul, five of seven DHF patients without shock demonstrated increased consumption of fibrinogen without other haemostatic abnormalities. These authors were unable to demonstrate the marked increase in fibrinogen synthesis that usually accompanies severe disseminated intravascular coagulation (DIC). Since euglobulin clot lysis times were normal and FDP levels only two to four times normal, the authors concluded that DIC did not contribute to shock and that, in the cases studied, heparin therapy was not justified. Funahara et al. speculated that the absence of FDP in children with low fibrinogen levels suggests a rapid clearing of FDP, and that the existence of low alpha 2 antiprotease levels suggests accelerated lysis of haemostatic plugs, which might be expected to facilitate bleeding. However, Cuban workers interpret decreased plasminogen levels simply as the normal physiologic response to the activation of fibrinogen.

Finally, there may be important age-related differences in haemostatic mechanisms in dengue infection. Srirkaikul et al. presented data for three adolescent Thai males who presented with profound shock, gastro-intestinal bleeding and multisystem dysfunction during dengue infection. After stormy courses that included heparin therapy, two patients died. A diagnosis of DIC was made in all three cases based on thrombocytopenia, generalized haemorrhage and one of the following: profound depression of serum fibrinogen levels, marked elevation in euglobulin lysis time or marked increase in FDP. Both fatal cases demonstrated fibrin thrombi on autopsy. In the Cuban DHF/DSS epidemic of 1981, where all age groups were equally at risk of a secondary dengue 2 infection, hospitalized adults more frequently than children had severe bleeding without elevated haematocrit readings, pleural effusions or other evidence of vascular permeability.

1.5 Complement Consumption

Although not formally recognized as part of the DHF syndrome, consumption of complement is always found and can be considered essential to its diagnosis and ultimately to an understanding of its pathophysiological mechanisms.

The pioneering work on complement was that of Russell et al. and Nishioka, who studied patient materials collected in 1967. Their findings were confirmed by Phanichyawat et al. These authors documented, in DHF patients with and without shock, significant decreases in levels of serum C3 and, in some instances, of C4. Suvatte et al., the collaborative World Health Organization (WHO) study and the successor studies of Bokish et al. have provided evidence that complement is activated by both the classical and alternative pathways in children who experience a secondary dengue infection and in infants during an initial dengue infection. C3, C3 proactivator, C4 and C5 levels were below normal in acute phase sera of DHF patients at all grades of severity. In addition, when analysed using the Children's Hospital grading system (grades I, II, III, IV), there was a severity-related linear reduction in these four proteins in acute phase sera. These losses could not be attributed to transudation because the severity-related reductions in their plasma values exceeded those of transferrin. Furthermore, C3 levels measured in pleural effusions in four patients were 30-62 per cent below concurrent serum values. Low levels of complement proteins were not found in 13 Thai children hospitalized with various viral and bacterial infections. Furthermore, normal C3 levels were observed in children ex-
C3 and Clq metabolism was studied in 17 and seven patients, respectively, using $^{125}$I-C3, $^{125}$I-Clq and $^{131}$I-IgG as markers of extravasation of large molecular weight proteins. Twelve patients with grades III and IV eliminated an average of 2.6 to 3.5 per cent of the C3 plasma pool per hour during a seven day period of hospitalization as compared with 1.9 per cent per hour in controls. The maximum period of increased catabolism was during shock. Similarly, the fractional catabolic rate of Clq metabolism varied between 3.8 and 8.3 per cent of the Clq plasma pool per hour as compared with 2.7 to 3.4 per cent per hour in controls. While a small amount of C3 activation may be attributed to the alternative pathway, it seems likely that changes in Clq catabolism reflect the fractional increase in complement activated by antigen-antibody complexing which has been found to be 1.4-3.1 fold.

From these studies it can be surmised that significant amounts of C3a and C5a anaphylatoxins are generated in DHF. These have the ability to stimulate mast cells to release histamine. A normal 20kg individual circulates 1.2g of C3, which potentially could release 44mg of C3a. This means that children might release up to 35mg of C3a or 4.8 million minimal wheal and flare doses from histamine release. As cited below, histamine production in DHF is apparently increased, although a central role for histamine as the mediator of vascular permeability and shock has never been established.

1.6 Vascular Permeability Mediators: Kinin, Histamine

Considering the importance of the problem, the number of studies designed to identify the vascular permeability mediator(s) in DSS is rather paltry. In 1977, Tuchinda and colleagues described the excretion of histamine in the urine of 12 children, aged four to twelve years. All had a clinical diagnosis of DHF with serological evidence of a recent dengue infection, two grade I, four grade II, five grade III and one grade IV. Total histamine in the urine of the grade IV case was five-fold higher than the mean in 12 control patients. There was a progressive increase in free and total histamine excretion according to severity of disease. The day of collection of urine and its relation to shock, if present, was not stated.

As pointed out by the authors, histamine might reflect C3a and C5a activity or its release from damaged platelets, both of which occur and have been related to disease severity in dengue.

A single study has been made of the plasma kinin system in DHF. Eleven shock patients studied in July 1972 were included. Assays for bradykinin, prekallikrein inhibitors (KI) and factor XII were performed on frozen serum shipped to Boston. Studies were carried out to measure activation of the kinin system at the several points indicated:

 inactive factor XII
 |
 active factor XII
 |
 prekallikrein --- kallikrein inhibitors inactive

 kininogen --- bradykinin* --- inactive

*assayed.

Prekallikrein and factor XII activities were significantly depressed prior to the onset of and during shock while kallikrein inhibitor activity was low prior to shock, normal during shock and then low again. Bradykinin concentrations showed no change in the pre-shock, shock or post-shock phases of illness. Although there was significant evidence of activation of key substrates in the kinin system without evidence of the consumption of kallikrein inhibitors and elevation of bradykinin concentrations, the authors concluded that increased vascular permeability was not mediated by the kinin system. They reasoned that depressed plasma levels of prekallikrein might be due to extravasation and
acute liver damage, while factor XII depression might reflect activation of the complement system. That being said, however, direct measurement of increased bradykinin in man using assay systems available in 1972 was difficult since kinase inactivators are abundant and assays for bradykinin breakdown products were not available.

An interesting possible vascular permeability agent is endotoxin, the features of shock and haemorrhage in viral infections bearing many similarities to endotoxin shock and gram-negative sepsis. Using the limulus amoebocyte lysate test (LALT), Usawatanakul and others assayed defervescence or shock phase blood from 57 DHF patients for presence of endotoxin. Endotoxaemia was detected on the day of maximal vascular permeability or shock in 50 per cent of cases. Non-shock DHF cases circulated endotoxin less frequently, although the difference, compared with shock cases was not significant. None of 20 controls circulated endotoxin. The authors conclusion is that endotoxin is not the principal cause of shock because it was not found in every shock case. None of 46 LALT-positive patients had bacteraemia, gram-negative or gram-positive. Whether, as surmised, endotoxin somehow leaks into the circulation from a damaged gut remains to be determined. The importance of endotoxin on performance and interpretation of the Clq deviation test should be studied (see below).

1.7 Immune Complexes

A number of authors have searched for immune complexes in DHF. In the WHO collaborative study, Clq precipitation was detected which roughly correlated with disease severity, and Clq catabolism was elevated. Complexes could not be visualized on the surface of patient leucocytes by the fluorescent antibody (FA) test. Using a direct measure of immune complex binding of Fc receptor-bearing cells, Theofilopoulos et al detected complexes in 62 per cent of DHF cases. The frequency of detection correlated with disease severity. More recently, Petchia and Saelim detected immune complexes in 47.5 per cent of DHF patients using a platelet aggregation technique. A novel assay system was described by Boonpucknavig et al, who studied mononuclear leucocytes obtained from 62 DHF patients and 37 controls. Unfixed lymphocytes were stained with anti-dengue, anti-IgG or anti-C3 separately or in combination. Dengue antigen and C3 were discovered on the surface of 0.6-2.1 per cent of cells, beginning before shock and peaking on the day of shock or subsidence of fever. Since dengue antigen was also found on the surface of IgG staining cells, the authors surmised these were B lymphocytes; rigorous proof that these were not K cells or monocytes was not provided. The percentage of Ig-bearing cells during acute illness was around 20 per cent. The authors found no correlation between the number of dengue antigen-bearing cells and the severity of clinical illness. Subsequently, Boonpucknavig and Striron were able to demonstrate binding of dengue antigen-antibody and dengue antigen-antibody complement to the surface of B lymphocytes (Fc receptor or C3 receptor-bearing lymphocytes) when studied in vitro.

Ruangjirachuporn et al, using several methods, measured immune complexes in sera from 80 DHF patients aged 2-14 years, all of whom had secondary antibody responses, using the ability of preformed complexes to bind Clq and thus prevent the agglutination by Clq of IgG coated latex particles. As in their earlier work, the authors found immune complexes before shock (or subsidence of fever), which peaked on the day of shock and one day later. The nodal day, after onset of fever, of peak immune complex formation was day five. A distinct trend was observed of increased concentration of immune complexes with increasing severity of disease. Anti-dengue stains revealed peak antigen absorbed to Raji cells prior to shock while anti-Ig stains showed peak values one day after onset of shock. There was a strong correlation between the percentage of anti-Ig positive cells and the severity of disease. By the Clq inhibition test, 84 per cent of 94 sera were positive. Using Raji cells, 75 per cent were positive for IgG and 58 per cent for dengue antigen.

Platelets from 107 DHF/DSS patients were studied by FA for attached IgG, IgM, C3 or dengue
antigen. Immunoglobulins and/or complement plus dengue antigen were detected in 11 cases\(^{(56)}\). There was no correlation of detection of immune complexes on platelets with disease severity. A similar study on 13 patients was reported by Phanichyakarn et al\(^{(57)}\), in which 12 patients showed platelets with C3 stain and there was a trend of more positives in the shock than non-shock group.

Further evidence for the formation of circulating immune complexes was furnished from kidney biopsies on 19 DHF patients with secondary dengue infections of grade I (one patient), grade II (nine patients), grade III (five patients) and grade IV (four patients)\(^{(58)}\). Biopsies were obtained 6-29 days after onset of fever. Although dengue antigens could not be detected in any case, deposits of IgG, IgM or C3 were observed in 11/19 glomeruli. On electron microscopy (EM), dense, spherical particles approximately 40 mm in diameter were found in the cytoplasm of mesangial cells, the lumens of glomerular capillaries and the cytoplasm of monocytes. Basement membranes showed irregular thickening of the lamina interna rara. There was proliferation and hypertrophy of the endothelial cells of glomerular capillaries and projection of elongated cytoplasmic processes into capillary lumens. These changes are typical of immune complex glomerular disease. Although the authors did not relate frequency or density of immune complexes as seen on EM to disease severity, they did observe immune complexes by IFA in 3/10 grade I and II cases compared with 7/9 grade III and IV cases. Crystalline arrays of spherical dengue viral-like particles were observed in the cytoplasm of monocytes, a finding which suggests dengue replication in this cell type.

In another tissue study, skin biopsies from 53 DHF cases were examined by IFA (59). No data were provided on disease severity. Biopsies were performed between days 3 and 13 after onset of fever, 40 of which were on or before day seven after onset of disease. Human C3 and/or IgM was observed in the walls of blood vessels in the dermal papillae of 11 cases. The fluorescence staining appeared in a granular pattern along the capillary walls, indicative of immune complexes. Dengue antigen was seen in the cytoplasm of mononuclear cells located in the dermal papillae outside dermal capillaries in 16 cases. Granular deposits of fibrinogen were deposited in capillary and dermal tissue in 30 cases, consistent with mild intravascular coagulation.

A final study, while not an absolutely definitive test for recognition of immune complexes, provides the strongest evidence of any assay system for the progressive increase in concentration of immune complexes with increasing severity of DHF\(^{(60)}\) (Figure 1). In this test, DHF sera are heated to release in vivo bound C1q from immune complexes. Radiolabelled C1q is added, followed by a sheep red blood cell anti-SRBC indicator, and binding or radiolabel measured. One hundred and ninety-three samples from 43 DHF patients were studied. Data were evaluated according to severity of disease. Sera from patients with grade IV disease exhibited the highest degree of C1q deviation, those with grade I the least. Serial serum samples of individual grade IV patients gave the highest values for C1q deviation on the day of shock compared to earlier or later serum samples. While the nature of the C1q reactive material was not definitely identified, partially purified material was able to activate complement in normal serum. The probability is that these materials were dengue immune complexes.

In summary, of eleven attempts to quantitate immune complexes in DHF/DSS, eight demonstrated a correlation between the frequency or concentration of complexes in the shock or defervescence phase and disease severity. Antigenemia, viraemia and corresponding cellular infection necessarily must be increased in severe (compared with mild) dengue infections. These data are consistent with the hypothesis of the immune enhancement of infection (see below).

### 1.8 Leucocyte Response

Early observations on dengue fever (DF) in Northern American adults documented a consistent leucopenia caused predominantly by a destruction of mature polymorphonuclear leucocytes\(^{(61-64)}\). Late
in the illness there were increased numbers of so-called Turck reaction cells (lymphoblastoid cells) and in convalescence a modest eosinophilia. By contrast, children with the DHF/DSS syndrome usually have a modest leucocytosis, with an early relative lymphocytosis followed by rising neutrophil and lymphocyte counts. As with DF, there is a "shift to left", with predominantly young PMN forms and degenerating mature PMNs.

Polymorphonuclear neutrophils (PMN) obtained from children and adults with dengue 2 infections in the Cuban DHF/DSS outbreak of 1981 showed a reduced ability to adhere to nylon filaments and to reduce nitroblue tetrazolium. Both of these functions are compatible with immaturity, presumably the result of destruction of mature PMNs.

In DHF/DSS, coincident with the day of shock or subsidence of fever, there is a marked increase in the absolute number and the per cent of atypical lymphocytes. Wells et al. found a 17-fold increase in atypical lymphocytes in 16 patients with secondary infection DHF/DSS compared with values for the same patients studied 30 days later. Boonpucknavig et al., studying 76 subjects with DHF/DSS, found a 54.8 per cent increase in atypical lymphocytes as compared with values for 33 age- and sex-matched controls. Thisyakorn et al. described the kinetics of atypical lymphocytes in 40 patients with DHF/DSS and secondary-type infections. Values rise rapidly one to two days before shock, peak on the day of shock and then remain for four days at the 30 per cent level. These cells have been described by Suvatte and Longsam (transformed lymphocytes. In 320 DHF/DSS patients, 20-50 per cent of total lymphocytes were lymphoblasts. The absolute number rose rapidly during the period five to seven days after onset of fever. Boonpucknavig et al. found approximately a three to four-fold increase in Ig-bearing cells peaking one to two days after onset of shock. In a later study, the same group described a three-fold increase in cells rosetting mouse erythrocytes (B
lymphocytes), which peaked on the day of shock, and an eight-fold increase in Ig-containing cells (plasma cells) that peaked on the same day. Wells et al.\(^{67}\) found a four-fold increase in null cells (plasma cells) on the day of hospital admission. Both groups described a 26-37 per cent reduction in the absolute number of T lymphocytes in the acute phase of DHF.

Cruz and others\(^{71}\), studying 50 children hospitalized in the 1981 Cuban DHF/DSS epidemic, found that the depression in T lymphocytes (E rosetting) was directly correlated with illness severity; 19 patients with grade I disease had a mean of 68.5 ± 9.9 per cent T lymphocytes; seven patients with grade II illness had 57.7 ± 10.6 per cent T lymphocytes; 13 grade III patients had 46.5 ± 11.6 per cent T lymphocytes; and 11 grade IV patients had 37.2 ±15.8 per cent T lymphocytes. These authors observed four per cent of lymphocytes to rosette mouse erythrocytes (B lymphocytes) while on the other hand Fc receptor-bearing cells and cells with surface Ig composed 20 per cent of the lymphocyte population (range 8-60 per cent). Interestingly, adults hospitalized with severe dengue infections (with secondary responses) had only modest reductions in T lymphocyte numbers.

Although the existence of T suppressor cells has received much speculative interest from authors\(^{72,73}\), apparently no studies have been made on human lymphocyte population subsets. However, Gilbreath et al.\(^{74}\) discovered that 61 per cent of 83 acute phase sera from DHF patients had cold-reactive anti-lymphocyte antibodies (ALA) of the IgM class capable of destroying autologous as well as allogeneic lymphocytes in the presence of complement. These demonstrated predominantly B but some T cell reactivity. ALA titers progressively decreased when measured 15 and 30 days after infection. The in vivo action of the ALA in DHF is unknown. Anti-lymphocyte antibodies are widely thought to be an important immunoregulatory mechanism. Presumably in secondary dengue infections they contribute to the destruction of plasma cells and the lymphocytes which have responded to dengue antigens.

Possibly related to the above phenomenon is the demonstration by Boonpucknavig and Udomsangpetch\(^{75}\) that 48 per cent of 170 DHF patients had antibodies in sera or antibodies which could be eluted from mononuclear leukocytes which were directed against the smooth muscle of rats. The authors speculated that dengue virus-infected cells might expose actomyosin-like proteins which elicit cross-reactive antibodies with smooth muscle. Alternatively, viral and smooth muscle antigens may show structural similarities.

### 2. PATHOGENESIS

Infectious disease pathogenesis is the outcome of the interaction of at least two organisms. In the case of viral infections, the virus has a limited genome but may exist in nature as a heterogeneous population; its gene structure may be subject to relatively rapid and significant change. The vertebrate host is vastly more complicated. Its genes programme cell receptors, and specific and nonspecific defence mechanisms; its homeostatic mechanisms are constantly perturbed by environmental inputs. As more observations are made on DHF, it becomes progressively clear that the pathogenesis of this disease is the result of a bewildering composite of extrinsic (viral and other) and intrinsic (host) factors. This section will review the evidence which links various extrinsic and intrinsic factors to risk in the causation of DHF/DSS. A final section will present a unified hypothesis of the pathogenesis of DHF/DSS.

#### 2.1 Site of Dengue Virus Infection in Man

Any insight into viral pathogenesis must begin by identifying the cell or cell types which support infection in vivo. Cells supporting dengue virus in man are not known definitively. Data from a number of lines of evidence suggest an exclusive or major role for cells of mononuclear phagocyte lineage. Most tissues obtained at autopsy have not yielded virus isolates when tested in tissue culture systems, presumably due to the relative lateness of death after illness onset and the high concentration
of neutralizing antibody in serum and tissues\(^{(76)}\). From patients with secondary infection DHF/DSS, virus has been recovered rarely from liver, lymph node, bone marrow and lung\(^{(77,79)}\). Virus recovery attempts from infants with fatal primary dengue infection are most successful, yielding dengue 1 and dengue 2 viruses from liver, spleen, thymus and lung in two cases\(^{(80)}\). By FA, dengue antigen has been found to be localized in Kupffer cells and in splenic, thymic and pulmonary macrophages. Skin biopsies from DHF patients have revealed dengue FA antigen in histiocyte-like cells in dermal papillae from 16 patients\(^{(76)}\). Dengue virus has been successfully recovered from peripheral blood leucocytes collected at the time of shock or defervescence from 76 of 332 DHF/DSS patients\(^{(81)}\). In a separate study, circulating leucocytes containing dengue antigen were identified as monocytes\(^{(80)}\). Particles resembling replicating dengue nucleocapsides have been seen in glomerular macrophages by electron microscopy\(^{(80)}\).

Findings in man have been reproduced in rhesus monkeys, where dengue virus replicates in histiocytes at the skin inoculation site, in macrophages in regional lymph nodes and in Kupffer cells and cells with macrophage morphology in spleen, lymph nodes and Peyer's patches. As virus spreads from tissue to tissue, it remains recoverable from previously involved sites. Thus, at the end of infection the number of cells involved is maximal. In these studies, intracellular infection was terminated quite abruptly seven to eight days after infection\(^{(82-84)}\).

2.2 Extrinsic Factors

**Previous immunologic experience**

The central extrinsic risk factor in DHF/DSS is the circulation of pre-infection antibody, whether from prior dengue (or other flavivirus?) infection or passively acquired from the mother.

Numerous hospital-based and prospective epidemiological studies have demonstrated extremely powerful correlations between secondary type antibody responses or previous dengue infections and an illness of dengue aetiology in children which requires hospitalization\(^{(83,85,86)}\). As disease
severity increases in infants and children older than one year the correlation strengthens, so that in this age group secondary-type antibody responses approach 100 per cent in dengue shock syndrome\(^{(9)}\). Critics have noted that DHF/DSS does not occur with all secondary dengue infections\(^{(92,93)}\). This is true; in Thailand, only 0.5-2 per cent of secondary infections result in shock\(^{(78)}\). In some ecological settings, the high proportion of non-pathogenic secondary infections appears to be due to a requirement for specific sequences of dengue virus infections to produce shock\(^{(66)}\).

As discussed more fully below, the fact that not all secondary dengue infections result in DSS does not invalidate the observation that there is a significant association between dengue shock cases and secondary dengue infection in children one year and older. A Venn diagram (Figure 2) summarizes the situation. The following statements are substantiated by multiple observations (in children older than one year):

- Not all secondary dengue infections result in DSS.
- Nearly all DSS cases occur with secondary dengue infection.
- DSS rarely occurs with primary dengue infections.

**Sequential dengue infections**

The most completely documented longitudinal study of DHF/DSS concerns the collaboration between Bangkok Children’s Hospital and the SEATO Medical Research Laboratory (now the Armed Forces Research Institute of Medical Sciences – AFRIMS). This study has been carried out, with only a few interruptions, between 1962 and the present. For the first 20 years of this period, dengue 2 was the dominant serotype associated with secondary infection DSS. In 1970, based upon data from the 1962-64 period, it was postulated that dengue 2 strains possessed unusual virulence properties which were expressed in the presence of antibody\(^{(33,84)}\). This phenomenon was documented in a prospective population-based study at Rayong, Thailand\(^{(96)}\), in which there was serological evidence for the infections of children with each of the 12 possible combinations of sequential infections with dengue viruses types 1-4 (i.e., DEN 1-2, 1-3, 1-4, DEN 2-1, 2-3, 2-4, DEN 3-1, 3-2, 3-4; DEN 4-1, 4-2, 4-3). While the most numerous infections were in the sequences DEN 2-1, 3-1 and 4-1, none of these resulted in shock syndrome. DSS was seen only with sequences ending in 2; DEN 1-2, 3-2 and 4-2\(^{(99)}\).

In the epidemiologically "clean" setting on the island of Cuba, a large epidemic of DHF/DSS was associated with secondary dengue infection occurring in the sequence DEN 1-2\(^{(95,98)}\). In a population almost completely without dengue infection experience since the time of World War II, a large "virgin soil" DEN-1 outbreak occurred in 1977-78\(^{(98)}\). Four years later DEN-2 invaded, creating an epidemic in which 300 000 dengue cases were reported, of which 116 000 were hospitalized. Probably around 10 000 had DSS\(^{(100)}\). The occurrence of DSS in children was well documented clinically\(^{(96)}\) as was its association with secondary-type antibody responses\(^{(97)}\). Further evidence that DSS occurred only in secondarily infected children was provided by the age distribution of shock and fatal cases (Figures 3 and 4). Children born after the DEN-1 epidemic (ages one and two years) were absent among such cases\(^{(95,96)}\). A similar sequence of infection may also have occurred in the 1928 Greek dengue epidemic which was accompanied by more than 1000 deaths\(^{(99,100)}\). A "silent" dengue outbreak occurred in 1927. There is evidence for major transmission of DEN-1 and minor transmission of DEN-2 in 1928. A significant fraction of residents of Athens alive at that time were shown to have serological evidence of two dengue infections\(^{(99,100)}\).

In summary, until 1981, the epidemiological circumstances which produced DSS most frequently were those in which secondary DEN-2 infections occurred in large numbers. Secondary DEN-2 has not occurred as a major epidemic phenomenon in the Caribbean basin outside Cuba in the modern era. This may be partially due to the fact that DEN-2 was widely seeded throughout the region prior to the introduction of DEN-1 in 1977.
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Figure 3. Distribution of 124 dengue shock syndrome cases by age, Cuba, 1981 (William Soler and Central Havana hospitals\(^{95}\))

Figure 4. Distribution of 72 fatal dengue shock syndrome cases by age, Cuba, 1981\(^{79}\)
As if to flaunt a perverse complexity, since 1984 DSS in Thailand has been associated at high frequency with secondary DEN-3 and DEN-4 infections (C.H. Hoke, personal communication). DEN-3 was highly endemic in Indonesia in the mid-1970s and was associated with hospitalized cases in Malaysia in the 1970s and 1980s [101-103], but the sequences producing these outbreaks are unknown.

Two recent prospective studies add to an understanding of mechanisms governing infection enhancement during sequential infections. Eckels et al [104] studied the infection enhancement of DEN-2 virus pre-immunization using sera obtained from 75 DEN-2 vaccine volunteers. In this group, 90 per cent of yellow fever immune seroconverted to the live attenuated DEN-2 vaccine while in susceptibles only 61 per cent seroconverted [105]. When tested, 1/14 non-immunes, but more than one-half (32/61) of the yellow fever vaccinated subjects, had DEN-2 infection enhancing antibody at a titre of 1:2.5 or higher. The geometric mean DEN-2 plaque reduction neutralizing antibody (PRNT) titres were 1:320 in the group with pre-infection enhancing antibodies and 1:30 in the group without. The circulation of DEN-2 infection enhancing antibodies was correlated with higher rates of vaccine "takes". Although yellow fever antibodies have not been implicated as a risk factor for DSS, what about non-dengue flavivirus antibody? Despite the effect of 17D yellow fever vaccination on DEN-2 vaccine "takes", no case of DSS has been attributed to a non-dengue flavivirus-dengue virus infection sequence. Dengue infections in U.S. servicemen acquired in Viet Nam were mild [106]. Of possible relevance is the observation that human JE antibody acquired from natural infections failed to enhance DEN-2 infections in macrophage cell lines [107]. When immunized by multiple injections, a wide variety of flavivirus antibodies raised in laboratory animals has produced infection enhancement of DEN-2 in cell culture [108].

Passively acquired dengue antibody

For its pathogenetic insights, the most interesting risk factor for DHF/DSS is passively acquired dengue antibody of maternal origin. The existence of this risk factor was surmised from two observations: (i) the consistent association of DHF/DSS in infants less than one year of age with primary dengue infections [33], and (ii) the peculiar age distribution of these cases, few occurring in the first three months of life, cases peaking at seven to eight months and incidence falling nearly to zero by the end of the first year of life [33,34]. In addition...
to Thailand, DHF/DSS in infants has been documented in Indonesia\(^{109}\), Cuba\(^{79}\), and Burma\(^{110}\). This phenomenon (primary infection DHF/DSS) appears to be restricted to DHF/DSS epidemic or endemic areas. The pathophysiological features of primary and secondary infection DHF/DSS seem to be identical; thrombocytopenia, increased vascular permeability, complement activation, mild DIC, and in severe cases, shock\(^{32,34}\).

A recent study by Khks \textit{et al}\(^{111}\) provides the strongest evidence to date that maternal antibody is a risk factor for DHF/DSS. These authors studied 13 infants hospitalized with DHF at Bangkok Children's Hospital, from each of whom DEN-2 virus was recovered during illness. Mothers were bled during their infant’s illnesses and again two weeks later. In each instance, the mother circulated multitypic dengue antibodies, derived from two or more remote dengue infections. The IgM/IgG ratios indicated that no mother experienced a dengue infection at the time of her infant's infection; each was solidly immune. It was surmised that maternal serum antibody was in all likelihood the same as the placental antibody transferred four to twelve months earlier. Each maternal serum produced infection-enhancement with DEN-2 virus recovered from her sick infant. Further, the titres of DEN-2 plaque reduction neutralizing (PRNT) antibody and the DEN-2 enhancing antibody correlated with the age of the infant when onset of DHF occurred. For example, infants born to mothers with low DEN-2 PRNT\(_{50}\) titres (i.e. less than 1:100) developed DHF at four, six and eight months, times which were quite close to the predicted time of disappearance of DEN-2 neutralizing antibody (titres of less than 1:1) based upon an assumed half-life of maternal IgG of 35 days. Babies born to mothers with high titred DEN-2 neutralizing antibody, i.e., 1:2000-8000, did not develop DHF until 11 to 12 months of age, a time also consistent with the disappearance of DEN-2 PRNT. All babies had DEN-2 enhancing antibody titres which were two- to ten-fold higher than those of neutralizing antibodies. DEN-2 infections in these infants demonstrably occurred in the absence of neutralizing and the presence of enhancing antibodies.

**Viral virulence**

The association of DSS cases with DEN-2 infection in Rayong in 1980, but the absence of such association with other dengue types, clearly implies a virulence phenomenon associated with DEN-2. It is not yet clear whether this phenomenon is governed by attributes of the antibody residuum from previous infection(s) or other factor(s). Nonetheless, "DEN-2-ness" might be a virulence attribute not shared by other dengue viruses in Thailand, at least prior to 1984. While the absolute number of secondary DEN-2 infections with Caribbean DEN-2 strains in recent years can only be guessed at, there must be some (outside Cuba); yet DHF/DSS in this population has been virtually nil. This is in contrast to the high frequency of DSS associated with secondary DEN-2 infection in Cuba in 1981, the responsible DEN-2 strain presumably being introduced from outside the region. It seems possible that Caribbean and Thai DEN-2 strains differ in their DEN-2 "virulence” properties when infecting individuals with circulating dengue antibody.

Morens \textit{et al}\(^{112}\), studying 13 DEN-2 strains isolated in 1980 from Bangkok children with disease of varying severity, observed significantly higher growth in human monocytes of strains from severe cases compared with virus recovered from children with mild disease. Recent preliminary data from Kliks (personal communication) suggest that DEN-2 viruses grow better than DEN-1, -3, or -4 viruses in human monocyte cultures supplemented with enhancing antibody. Virulence, therefore, may reside in the efficiency of replication by DEN-2 viruses in infected mononuclear phagocytes.

Some authors have suggested that viral virulence is a risk factor for DHF/DSS independent of pre-infection antibody status\(^{93,115-117}\). Such assertions are at variance with data from a large number of prospective studies on DHF/DSS epidemics\(^{13,85-99}\). The evidence for primary viral virulence is meagre, being based upon hypotheses unsupported by observational data, such as that \textit{Aedes aegypti} selects for virulent strains\(^{113}\); or that virulent strains come from monkey jungle cycles\(^{110}\);
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or is based on data from studies in which key cases have not had dengue as an established cause of illness\(^{113}\); or on data from cases which did not satisfy the diagnostic criteria for DHF/DSS of hypovolaemia plus thrombocytopenia\(^{113}\); or is based on cases which were defined as primary or secondary by haemagglutination inhibition (HI) tests done on single acute phase sera only\(^ {117}\). Without the use of IgM/IgG ratios or the neutralization test, a single HI test does not accurately characterize all primary or secondary antibody responses. Approximately ten per cent of documented secondary dengue infections have low or absent HI antibodies in acute phase serum specimens\(^ {65}\), a phenomenon which may exemplify the negative phase of a secondary response, i.e., the in vivo precipitation of antibodies by high concentrations of antigen.

Another element in the critique of the association between DSS and secondary-type antibody responses has been the assertion that the frequency of initial dengue infections in DHF/DSS endemic areas is so high that all children are dengue-immune after one year of life. This, it was reasoned, is why a high fraction of dengue infections after this age are secondary\(^ {90}\). The study cited as typical was done on groups of children resident in various districts of Rangoon in 1974\(^ {118}\). In this study, both chikungunya and dengue HI antibody prevalence rates were observed to be 100 per cent for the age group of one to five years, values never reported before or since and rather indicative of test error. The same group in the same year performed a prospective study on a cohort of 86 children, aged 0-2 years, observing a dengue infection rate of only 23 per cent, leaving 77 per cent of this age group still susceptible\(^ {116}\).

At any rate, the assertion that DSS and secondary infection are related merely in a random fashion has long been laid to rest, since pre-outbreak antibody prevalence rates in all children aged 1-14 years in DHF/DSS endemic and epidemic areas has averaged 50 per cent or less, while the association of shock with secondary-type antibody responses at every age over one year has been nearly 100 per cent\(^ {46,69}\), yielding relative risks associating shock and secondary infection as high as 502\(^ {90}\).

Experimental studies

**Antibody dependant enhancement of dengue infection in mononuclear phagocytes.** Data which seem to be relevant to the DHF/DSS phenomenon have been developed from four studies in experimental biology:

- Bone marrow or peripheral blood leucocyte (PBL) cultures prepared from dengue-immune human beings or rhesus monkeys were found to be permissive to dengue infection while similar cultures from non-immune individuals were not\(^ {119,120}\). The PBL which supported dengue infection was shown to be a monocyte\(^ {121}\).

- When dengue antibody is added to non-immune bone marrow or PBL cultures at dilutions which do not neutralize viral inocula, cells become permissive to dengue virus infection\(^ {122}\). This phenomenon requires interaction between the Fe receptor and Fe portion of an IgG-dengue virus complex\(^ {122}\). There is evidence that antibody brings the virus into contact with other cellular receptors resulting in internalization of virus and cellular infection\(^ {123}\).

Similar experiments have been successfully duplicated in a number of laboratories using a number of dengue viruses and a wide range of flavivirus immune sera\(^ {126-135}\).

- In another experiment, susceptible rhesus monkeys infected individually with DEN type -1, -3 and -4 viruses were challenged with DEN-2 at intervals of six weeks to six months. When the viraemia levels in animals infected in the sequences DEN 1-2, 3-2 and 4-2 were compared with those in susceptible animals infected with DEN-2 only, the former group exhibited mean peak viraemia titres 11.7-fold higher than those in non-immunes\(^ {124}\).

- It was also possible to demonstrate enhanced viraemia in vivo in rhesus monkeys receiving
small amounts of human cord blood dengue antibodies intravenously and then infected with DEN-2. When compared with controls inoculated with non-immune cord blood serum, viraemia levels in experimental animals were enhanced 2.7 to 51.4-fold\(^{(125)}\).

Enhanced infection with dengue viruses by activated macrophages. In vivo observations suggest, and in vitro studies demonstrate, the existence of non-antibody mechanisms for enhancing dengue virus uptake by, and infection of, mononuclear phagocytes.

Pertussis vaccine treated dengue-susceptible gibbons inoculated with DEN-1 virus demonstrated enhanced viraemia compared with similarly infected susceptible control animals\(^{(136)}\). In a similar experiment, rhesus monkeys were inoculated intravenously with *C. parvum* or pertussis vaccine and then infected with DEN-2 virus (Haltstead, S.B. Unpublished). Animals inoculated with these lymphopoietic stimulating agents circulated dengue virus in the blood at higher titres than controls (Table).

While the mechanism by which viral infection is increased in monkeys *in vivo* is not known, the results are remarkably similar to those obtained in a series of experiments conducted by Hotta and associates\(^{(137-141)}\), who treated macrophages with a variety of bacterial and parasite cell wall components, eliciting macrophage activation and increased numbers of dengue-infected cells. The substances which produced non-immunological enhancement of viral growth include lipopolysaccharide\(^{(137)}\), phytohaemagglutinin\(^{(137)}\), bacterial cell walls from seven species of bacteria\(^{(138)}\), peptidoglycan subunits of *Staphylococcus epidermidis*\(^{(138)}\), a peptidoglycan polymer\(^{(146)}\), Bordetella pertussis toxin\(^{(139)}\), lipopolipidic derivatives of muramyl peptides\(^{(148)}\) and extracts of adult *Acaris* and *Parascaris* worms\(^{(141)}\). In all experiments, macrophage activation was accompanied by increased uptake of latex particles strengthening the supposition that increased dengue infection was due to an increased uptake of virions.

All of the observations cited above are consistent with a unitary mechanism governing infection of mononuclear phagocytes since both antigen antibody complexes and a variety of polysaccharides attach to macrophage plasma membrane receptors resulting in activation phenomena characterized by increased motility, increased numbers of Fc and other cell membrane receptors, and increased phagocytic and pinocytic activity.

Evidence for macrophage activation in dengue haemorrhagic fever/dengue shock syndrome. As hypothesized below, phlogistic and vascular permeability factors may be generated by activated mononuclear phagocytes. When mononuclear phagocytes are activated, a host of enzymes is released. The proof of such activation requires the demonstrated production of an enzyme specific to this cell system. This may be difficult. Following the observation that serum acid phosphatase levels were elevated in DHF, Lam et al\(^{(142)}\) studied tartrate-resistant acid phosphatase blood levels in ten children with DHF (severity unstated). Enzyme

### Table. Non immunologic enhancement of dengue 2 virus infections in rhesus monkeys - Viraemia (PFU/ml) on days after infection

<table>
<thead>
<tr>
<th>Pre-infection</th>
<th>Monkey</th>
<th>Day after infection</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Pertussis Vacc.</td>
<td>UH13</td>
<td></td>
<td>870</td>
<td>870</td>
<td>9800</td>
<td>9800</td>
<td>1900</td>
<td>50</td>
<td></td>
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<tr>
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<td></td>
<td>34</td>
<td>13,000</td>
<td>37,000</td>
<td>157</td>
<td>1800</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>UH14</td>
<td></td>
<td>130</td>
<td>25,000</td>
<td>5000</td>
<td>2,650</td>
<td>17</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>UH15</td>
<td></td>
<td>250</td>
<td>1,000</td>
<td>184</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>UH11</td>
<td></td>
<td>117</td>
<td>480</td>
<td>870</td>
<td>870</td>
<td>670</td>
<td>100</td>
<td></td>
</tr>
<tr>
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<td>UH12</td>
<td></td>
<td>150</td>
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<td>750</td>
<td>420</td>
<td>100</td>
<td>160</td>
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</tr>
<tr>
<td>Control</td>
<td>W92</td>
<td></td>
<td>150</td>
<td>850</td>
<td>480</td>
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<td>100</td>
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<tr>
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<td></td>
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<td>500</td>
<td>300</td>
<td>34</td>
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</tbody>
</table>
levels were compared during the acute and convalescent phases of disease. The serum levels of tartrate-resistant acid phosphatase were nearly two times higher during the acute phase than in the convalescent illness stage. To date, the only known site of synthesis of this enzyme is the osteoclast, and enzyme levels are elevated in normal children and adults following bone fractures, in osteoclastic bone tumours and in tumours metastasized to bone. There being no obvious reason for increased osteoclastic activity in DHF, the authors speculated that a small population of macrophages might produce this enzyme. Further studies are needed.

Viral epitopes, antibody idiotypes and infection enhancement. When various lots of dengue and other flavivirus polyclonal antisera are mixed with uniform concentrations of a single dengue virus seed lot and added to fixed concentrations of mononuclear phagocytes, the highest dilution which reproducibly causes infection enhancement varies widely. Clearly this phenomenon must reflect differences in the number of immune complexes formed and correspondingly large differences in the number of dengue-reactive antibody molecules in these antiserum lots. Since the epitopic composition of dengue strains differs markedly there must be a corresponding heterogeneity of idiotypes in monoclonal antibody constituents of antisera. Immune complex generation in sequential dengue infections might be driven by epitopes on the second infecting virus or by idiotypes raised to epitopes on the first infecting virus.

Monoclonal antibodies provide a powerful tool with which to study these phenomena. The relevant studies to date are by Halstead and Morens and colleagues. These workers studied infection enhancement of a small battery of DEN-2 strains by a small library of monoclonal antibodies which were raised to DEN-2 New Guinea C strain. The studies demonstrated the presence and absence of type and group-specific infection-enhancing epitopes on the seven strains analysed. When extended to a battery of 19 monoclonal antibodies raised to DEN-4 4328-S strain, an even greater number of different epitopes was discovered. Infection-enhancing epitopes were heterogeneously distributed on seven DEN-2 strains. Importantly, monoclonal antibodies demonstrating DEN-4 specific reactivity in HI and neutralization tests produced infection enhancement with DEN-2 strains. When 17 DEN-2 viruses isolated in 1980 from patients with disease of varying severity were tested for infection enhancement using eight different DEN-4 monoclonal antibodies, those strains from mild syndromes were non-reactive, while strains from grade II and III patients were markedly reactive in infection enhancement tests.

2.3 Intrinsic Factors

The outcome of parasitism might be partly controlled by the products of the host's genome as well as by environmental factors producing phenotypic changes in the host. A genetic contribution to pathogenesis is suggested by evidence that differences in the frequency of severe outcome of infection are related to ethnic group, sex, and age, while a slight effect is correlated with HLA types or ABO MN blood groups.

Sex

A greater occurrence of shock syndrome and fatal outcome has been observed in females compared with males who acquired dengue infection in Thailand. An essential restriction was age of four years or more. When dengue infection rates were measured in the open population, no significant differences between males and females or children younger or older than four years were observed. Greater numbers of females among shock syndrome cases have also been observed outside Thailand. Usually, however, case reporting formats mix together all syndromes regardless of severity and age, effectively diluting the risk factor of femaleness with a larger number
of mild cases in which males are slightly dominant(33). Not only is the fraction of males slightly higher than females in the normal population, but most infectious diseases produce overt illness and death in males more frequently than in females. That dengue differs in this respect is of great interest pathogenetically, and the casual attitude of those who analyse the epidemiological data from DHF/DSS outbreaks is to be deplored.

**Age**

The 1981 Cuban epidemic provided the first modern opportunity to study the effect of age on the syndrome(s) associated with secondary DEN-2 infection. It had been well established that the population up to the age of 40 years was completely susceptible to dengue viruses at the onset of the DEN-1 outbreak in 1977(98). The 1977 epidemic affected all age groups equally(98). It may be surmised that DEN-2 also affected all age groups equally. Because of the strong likelihood of similar age specific secondary DEN-2 infection rates, cases reported by age, or age specific hospitalization rates or symptoms and signs described by age can be contrasted with some degree of confidence. Examination of figure 3 suggests that susceptibility to shock syndrome is relatively constant between the ages of 4 and 12 years, but steadily declines through the teenage years(95). Although Cuban adults were hospitalized, 101 deaths occurred in children of less than 15 years, and 57 in persons older than 15 years. This produces significantly higher fatality rates for children (3.83/100 000) than for adults (0.77/100 000)(96). The most striking observation in adults was the low frequency of dengue shock syndrome and the high frequency with which haemorrhagic phenomena were associated with severe disease(41).

**HLA and blood groups**

Single studies on the distribution of 35 HLA A and B types of lymphocytes and ABO MN blood groups have shown low frequency associations of markers with DSS. Significant associations occurred with three of 34 HLA groups, and blood group M occurred in 29 per cent of DHF patients compared with 18.5 per cent of controls(149,150). These associations appear to occur at about the five per cent level expected by chance and hence may not provide significant markers of risk to DSS.

**Chronic diseases**

Kouri and others(96) have described an increased association between DSS or dengue death and a history of asthma in the individual or family, a family history of diabetes, or anaemia in the individual. Control values and careful statistical evaluation were not given. An example of these associations in 88 "shock" cases in children is: asthma in the patient - 21.5 per cent, and asthma in the family - 36 per cent, compared with an incidence of paediatric asthma in Cuba of 11 per cent(95).

**Immune response**

Little is known of the immune response which eliminates dengue infection in vivo. For over 20 years the author has looked for killer cells capable of lysing dengue-infected target cells in experimentally infected rhesus monkeys. While the standard correlates of cell mediated immunity can be demonstrated in peripheral blood lymphocytes two to three weeks after a dengue infection(151,152), circulating killer cells, which have been observed seven to nine days after infection, are detected inconsistently. These cells have not been identified. Recently, Kurane et al(153,154) reported human natural killer cells (Leu 11 and T3 cells) and antibody dependent cell mediated cytotoxicity (leu 11 cells) which kill DEN-2 infected Raji cells (human B lymphoblastoid cell line).

**Nutrition**

There have been numerous anecdotal reports associating severe dengue syndromes in children with those individuals who are unusually well nourished(94) or who live in residential areas predominantly populated by middle or upper income groups (Them, personal communication). As yet, a well designed study on the relative risk of
DSS in well nourished children versus those with stage I, II or III protein-calorie malnutrition (PCM) has not been published. Sugiyanto et al. described studies on 58 DSS cases seen in Jogjakarta in 1979-80. No case of shock was seen in children at or below 60 per cent of the Harvard standard; no case of shock was seen in children with PCM II. However, no obvious difference in frequency of DSS was observed between well nourished children and those classified as having PCM I. No controls were provided. Patients were weighed during the acute illness.

In Cuba, 92.7 per cent of DHF cases were in the average and above average weight and height groups for age. The below average nutritional status group was arbitrarily defined as the lower ten per cent of the childhood population (Kouri, G., personal communication).

2.4 Pathogenesis Hypothesis

The goal of studies on pathophysiology and experimental pathogenesis is to understand the underlying or basic mechanisms of disease. It is hoped that improved treatment might result, or that meaningful insights might be transposable to other systemic viral diseases, viral exanthems or haemorrhagic fevers. In this context, a meaningful and testable hypothesis of the pathogenetic mechanisms of dengue is not a mere intellectual exercise, but a probe for many biological systems.

A hypothesis of the immune enhancement of infection has been described in a number of publications with relatively little change over the past ten years (19,78,91,123,155-159). It is founded on one untested (but testable) assumption, that the severity of systemic viral disease is related directly to the number of cells infected. In the case of human dengue infections, the hypothesis also depends on evidence, still far from exhaustive, that cells of mononuclear phagocyte lineage are important, perhaps exclusive, hosts of dengue viral infection.

It is hypothesized that antibody plays a dominant afferent role in driving dengue infection in opposite directions; more or fewer cells infected. When heterotypic neutralizing antibodies are present in undiluted serum, some or all viruses are neutralized and viral infection and disease are minimized. In the absence of heterotypic neutralizing antibodies, dengue monotypic or (in infants) low titre dengue multitypic antibodies form complexes with dengue viruses which infect mononuclear phagocytes with enhanced efficiency. As noted above, the number of epitopes shared by sequential pairs of infecting dengue viruses may be an important mechanism regulating the amount of infection enhancement and disease severity.

Since DSS occurs with equal virulence in the midst of a brisk secondary antibody response and in the early phase of a primary antibody response, it is not possible that immune complexes drive the efferent events in DHF/DSS. Several efferent phenomena have been identified and discussed above under extrinsic and intrinsic factors. There continues to be evidence that sex and nutritional status are related paradoxically to disease severity. These observations are the basis for the assumption that the integrity of the immune response is the predominant force controlling efferent phenomena in dengue. Although not reviewed extensively in this chapter, little is known about how dengue virus infected cells are eliminated. The suspicion is that this is a cell-mediated event. Important to the immune enhancement hypothesis is the assumption that, as the cellular attack on virus-infected macrophages is mounted, there occurs an initial activation phase. It is during this phase that pharmacological factors are released which produce increased vascular permeability, complement activation, altered haemostasis and shock. Of possible relevance to an activation event, it has been observed that there is decreased reticuloendothelial clearance after shock (10).

There is an elegance to a central pathogenetic role for macrophage phlogistic factors in DSS, because of possible pathogenetic unity with other viral haemorrhagic fevers. Extensive macrophage infection occurs in most viral haemorrhagic fevers; virus infection in some instances could be a macrophage activator. It is probable that the kinetics of the cell mediated immune response do not differ in primary or secondary dengue infection.
Therefore, shock related to immune clearance would occur on the same day after onset of fever in primary and secondary dengue infection. Disease severity is simply related to the number of macrophages infected and the immune competence of the host.

Just how ethnicity or other generic mechanisms control disease severity (as suggested by fragmentary data from Thailand and Cuba) remains to be determined. One plausible mechanism (of host or virus) is regulation of the efficiency of replication of dengue virus once it enters target cells.

All these questions, and many more, challenge those who continue to investigate this devastating and needless disease.

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Pathophysiology and Pathogenesis of DHF


Chapter 7
Dengue Viruses
by
Prasert Thongcharoen
Chantapong Wasi
Pilaipan Puthavathana

1. INTRODUCTION
DENGUE viruses, previously known as members of Group B arboviruses, are members of the genus Flavivirus within the newly established family of Flaviridae. Since the term “arboviruses” is used in a purely ecological sense to indicate the large group of viruses with biological cycles involving arthropods and vertebrates, Flavivirus meets the requirements for both biological and molecular structure.

Figure 1. Dengue 2 virus (size = 40 nm)

Source: Photograph provided by Mrs Surang Sa-Nguan Wong, Virus Research Institute, Ministry of Public Health, Thailand
The family Flaviviridae comprises a single genus with two possible members. Currently, the genus Flavivirus consists of a group of some 70 closely related human and veterinary viruses causing asymptomatic infections as well as many serious illnesses. Most species of Flavivirus are also arboviruses in the biological sense, although some evidently lack arthropod vectors. Arthropod-transmitted flaviviruses replicate in the arthropod hosts as well as in the vertebrate hosts. Human Flavivirus diseases are caused by diverse and complex pathogens with different viruses exhibiting marked tissue tropism. Many are neurotropic viruses such as Japanese encephalitis virus, St. Louis encephalitis virus, Murrey Valley encephalitis virus; others, such as dengue viruses, replicate preferentially in host macrophages, whereas yellow fever virus is usually viscerotropic.

2. STRUCTURE

Flaviviruses are enveloped viruses, about 45 nm in diameter, containing infectious single stranded linear RNA of molecular weight about $4 \times 10^6$, which is capped at the 5' end but lacks a poly(A) tract at the 3' end. The spherical particle displays icosahedral nucleocapsid symmetry; possesses lipid-containing ether-sensitive envelopes which comprise a single species of glycoprotein E that is embedded in host-derived lipid; and is associated with a membrane-like protein M which surrounds the RNA encased in the core protein C. The gene sequence commences 5'-C-M-E... Both the replication strategy and the mode of morphogenesis are distinct in several respects from the replication process of the well-characterized members of the Togaviridae in which the Flavivirus genus was formerly classified.

Dengue virus-infected cultured cells or mouse brain tissues have been analyzed under the electron microscope for morphogenesis. Complete enveloped particles are located in the cisternae of the rough endoplasmic reticulum. As no budding has been observed, it has been postulated that core structures might acquire an envelope by condensation of reticulum membrane. Virions are predominantly released from the organelles by reverse pinocytosis.

There are four distinct types of dengue virus, namely dengue virus type 1 (DEN-1), dengue virus type 2 (DEN-2), dengue virus type 3 (DEN-3) and dengue virus type 4 (DEN-4). Dengue virions appear, under the electron microscope, as spherical particles approximately 50 nm in diameter with small particles of seven nm on the surface. Like other flaviviruses, DEN viruses are inactivated by trypsin, chymotrypsin, papain and pancreatic lipase. DEN viruses resist treatment with ox bile for 15 to 20 minutes at a final concentration of 1:20, but they are completely inactivated by the same substance at concentrations of 1:15 to 1:5. The effect of pH on the virus particles depends on the suspending medium. Generally, the stability of DEN viruses is optimal at pH 8.0.

DEN viruses can be preserved in a frozen state at -70 degrees C, and for several years after suitable lyophilization. Human blood has been found to be infectious after storage in an ordinary refrigerator for several weeks.

DEN viruses are photodynamically inactivated by 1:100 000 neutral red and visible light, with a 10⁴-fold reduction in titre after 20 minutes. Exposure to 2000 ergs/cm of UV radiation for three minutes will totally inactivate $10^6$ FFU of purified DEN-2 virus in a protein-free medium. DEN viruses are readily disintegrated during equilibrium centrifugation in cesium chloride, but generally remain intact during rate zonal centrifugation through sucrose as well as equilibrium centrifugation in deuterium oxide-sucrose.

When the virus is propagated in cell cultures of mouse brain, progeny viruses, non-infectious haemagglutinins and soluble antigens are produced and released. It has been shown that the host cell origin of the virus affects the sedimentation coefficient and density. DEN-2 virions propagated in cell cultures have a sedimentation coefficient of approximately 205S, but when propagated in mouse brain show a sedimentation coefficient of 175S. The density of DEN-2 virions propagated in
suckling mouse brain ranges from 1.22 to 1.23 g/cm² by equilibrium centrifugation in caesium chloride, whereas those propagated in cell cultures range from 1.23 to 1.24 g/cm²

Depending upon the sedimentation rate, there are two kinds of haemagglutinins, namely, a rapid sedimenting (RHA) and a slow sedimenting (SHA) haemagglutinin. The optimum pH for both RHA and SHA is 6.2. The SHA was shown to have a doughnut structure of 13.4 to 14 nm in diameter with an accumulation of negative stain in a central zone five to seven nm in diameter

RHA of DEN-1 and DEN-2 co-sediment with the infectious virus in a rate zonal sucrose gradient forming a single peak

SHA particles sediment at 70S regardless of the host system of propagation. Equilibrium centrifugation in a caesium chloride or deuterium oxide-sucrose gradient does not separate RHA from SHA. Direct density determination of virus concentrates will result in a single peak ranging from 1.22 to 1.24 g/cm containing both RHA and SHA

An extended period of centrifugation in a 50 per cent potassium tartrate-30 per cent glycerol gradient can separate these two particles

As mentioned earlier, infectivity of all DEN viruses is destroyed by lipid solvents. DEN viruses are readily destroyed by shaking with ether. Tween 80 plus ether destroys DEN virions but leaves haemagglutinin (HA) activity intact in the form of SHA. Acetone effectively degrades virions, and converts virions into smaller slow sedimenting HA particles. Sucrose-acetone extraction has been used as a standard procedure to produce HA antigen for a routine serological test

A soluble antigen of complement-fixing type (SCF) is found in DEN virus infected mouse brain and serum of infected mice. The SCF is a small particle of seven nm, indistinguishable from the seven nm particles observed around disintegrating RHA. It is a non-structural protein, now called NS1 protein, with a buoyant density of 1.32 g/cm² and lacking HA activity. DEN-2 SCF has a sedimentation coefficient of approximately 4S. It is antigenically different from the virion and SHA. Urea, sodium dodecyl sulphate (SDS) and 2-mercaptetanol (2-ME), which destroy the serological activities and structural integrity of virions, do not inactivate non-structural SCF antigen

Immunoprecipitation of infected cells with antiserum prepared against the DEN SCF antigen has demonstrated that this antigen is equivalent to the non-structural glycoprotein GP46 or NS1. By molecular sieve chromatography, the virion, SHA and non-virion antigens of higher molecular weight can be separated from each other.

3. GENOME REPLICATION AND ORGANIZATION, AND PROTEIN

The sequencing of DEN-2 and DEN-4 genomic RNA has been achieved. The nucleotide composition for DEN-2-specified RNA is 33.2 per cent A, 21 per cent U, 25.3 per cent G and 20.5 per cent C. Codon usage is not random. Usage of codons containing the C-G dinucleotide is rare

Stem and loop structures at the 5' and the 3' extremities of the genome may be involved in replication and translation steps as well as in genome interaction with capsid protein.

The 40S RNA genome is the initial template for which a minus-strand complementary RNA is synthesised. Progeny genomic RNAs are synthesised from the minus-stranded template by a process involving replication, intermediates and replicative forms. The genomic RNA is the only viral RNA found in polysomes in DEN-infected cells.

A single open reading frame extending from nucleotide 97 to nucleotide 10 296 in the DEN-2 genome encodes 3391 amino acids. This suggests that the viral proteins are released by proteolytic processing to the precursor polyprotein. The 5' terminal one fourth encodes the structural proteins of the virus in the order C, prM(M), E. During the maturation of the virion, prM is cleaved to produce M. No M protein has been detected in infected cells as the cleavage should occur late in virus maturation. The non-structural proteins are encoded in the 3' three fourths of the genome in the order NS1, NS2A, NS2B, NS3, NS4A, NS4B,
NS5. The N-terminus of the major proteins of the DEN-2 virus has been identified by amino acid sequencing\(^{(19)}\).

Different virus-encoded or cellular proteases have been proposed for the proteolytic cleavage of the polyprotein precursor and are summarized in Table 1. The cleavage of prM seems to occur at a site specifically recognized by a protease in the Golgi apparatus. The N-termini of the glycoproteins prM, E, and NS1 are preceded by extended non-polar domains that serve as signal sequences for translocation of the proteins through the membrane of the endoplasmic reticulum and as spanning domains to anchor M and E in the lipid bilayer. A cellular signalase is involved in the maturation of the proteins. The cleavage of the other non-structural proteins after double basic residues could be catalysed by virus-encoded enzymes.

The DEN NS1 protein has been shown to be the complement fixing antigen. The functions of NS1 protein and of the hydrophobic NS2 and NS4 proteins remain unknown. NS3 and NS5 proteins contain short regions significantly homologous to sequences present in several RNA virus proteins that are involved in viral replication and/or transcription. Comparison of the characterization of the DEN virus specific proteins is summarized in Table 1.

The overall similarity in the entire sequence of all proteins between viruses belonging to the same group and between viruses of different groups in the Flaviviridae family has been analysed. The percentage of amino acid similarity between the DEN serogroup, yellow fever and the Japanese encephalitis serogroup ranges from 44 to 51 per cent while the percentage positional identity is 63 to 76 per cent within the DEN or the Japanese encephalitis complexes. Genetic variants of DEN-2 viruses show more than 90 per cent similarity within the amino acid sequence.

### 4. ANTIGENIC DETERMINANTS

DEN virus has been shown to contain at least five classes of antigenic determinant: group common reactive, subgroup reactive, complex specific, subcomplex reactive and serotype specific\(^{(20)}\).

Antigenic cross-reactivity among the members of the Flavivirus genus appears to involve a group-reactive antigen shared by all members. These broadly reactive determinants have been demonstrated by complement fixation, immunodiffu-

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**Table 1. Characterization of structural and non-structural proteins of dengue virus.**

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<tr>
<th>Proteins of dengue virus</th>
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<th>Non-structural</th>
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*Newly proposed term
**Function unknown

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fusion, neutralization and radioimmunoassay methods. Among members of Flavivirus, several complexes or subgroups have been established, namely, tick-borne viruses, the mosquito-borne-WN subset, the mosquito-borne-Spodweni subgroup, the NTA subgroup, the dengue subgroup and the viruses-with-no-known-vector complex\(^2\). The antigen which is responsible for such differentiation is a complex-specific determinant. Complex-reactive antigens can be demonstrated by complement fixation, immunodiffusion or neutralization. Furthermore, monoclonal antibody (mAb) studies have shown that an epitope which is contained within the variable region of the E glycoprotein is DEN subcomplex specific and common to DEN-1 and DEN-3\(^2\). Each virus within the complex can be identified as a serotype containing type-specific determinants, which will react only with homologous antibody and complex reactive antigen common to viruses within the complex. The differentiation of four antigenically distinct dengue serotypes is mostly accomplished by the neutralization test. However, individual strains of DEN virus show significant variation in the extent of the plaque reduction neutralization test (PRNT) by a single prototype reference immune serum and also in an oligonucleotide fingerprint study\(^2\). The E glycoprotein has multiple antigenic determinants and biochemical functions, including both cross-reactive and serotype-specific ones. A sixth class of epitope related to strain specificity probably also exists on the E glycoprotein\(^2\). A strain specific epitope was also demonstrated to exist in the NS1 (SCF) protein\(^2\). The E glycoprotein is antigenically reactive in common serological tests, e.g., haemagglutination-inhibition, immunodiffusion, neutralization, complement-fixation and radioimmunoassay.

Variation within the type determinants differentiates viruses within a serocomplex and strains of viruses that differ antigenically from the prototype. The protein molecule contains regions which are conserved (invariant) and shared by different flaviviruses. The region responsible for type-specific and complex-specific determinants is situated in unconserved (variant) portions\(^2\). Most of the determinants of the E or V3 proteins are type-specific; some are complex-reactive and a smaller number are flavivirus group-reactive. The nucleocapsid or V2 protein contains only flavivirus group-reactive antigenic determinants. Determinants which are responsible for haemagglutination-inhibition and neutralization antibodies are distinctive. This information leads to the explanation of temporal dissociation between neutralizing and haemagglutination-inhibition antibodies. Neutralizing antibody remains for years whereas haemagglutination-inhibition antibody declines in several weeks and plateaus for many years at a lower level\(^2\). It has been clearly shown that protection against homotypic secondary dengue infection by serotype-specific antibody is long-lasting and that the presence of circulating cross-reactive antibody may be responsible for in vivo enhancement of dengue viraemia which, in turn, might cause severe disease\(^2\).

5. MOLECULAR ANALYSIS OF DENGUE VIRUS ISOLATES

Genotypic variation among DEN viruses has been studied by means of different techniques allowing definition of the origin and dissemination of disease outbreaks. Oligonucleotide fingerprint analysis has been used successfully to differentiate viruses within a serotype of RNA Viruses, e.g. Indiana and New Jersey serotypes of vesicular stomatitis virus\(^2\), La Crosse viruses\(^2\), Influenza H1N1 viruses\(^2\), and St Louis encephalitis viruses\(^2\). In general, these studies indicate that viruses which cannot be differentiated serologically can easily be distinguished by their RNA fingerprints.

Oligonucleotide fingerprint analysis of 40S RNA species of DEN viruses isolated from several geographical areas has been reported. DEN-1 Pacific/South-East Asian strains exhibit little homology with Caribbean strains (20-30 per cent). It is interesting that the fingerprint map of Jamaican virus isolated in 1977 is very similar to African/Sri Lankan strains (50 per cent homology), suggesting an ultimate origin for this virus isolate\(^2\).
Although some virus strains of DEN-2 are serologically indistinguishable, differences can be clearly seen on mapping. Forty-one strains of DEN-2 have been classified into five genetic variants or topotypes:

- The Puerto-Rico - South Pacific topotype;
- The Burma - Thailand topotype;
- The Seychelles topotype;
- The Philippines topotype; and
- The Jamaica - West African topotype.

DEN-4 Caribbean virus isolated in 1981 showed 89 per cent homology with the virus strain isolated in 1980 from the Niue Islands in the Pacific, and showed only 37 per cent homology with the prototype studied.

Variation in one DEN-2 topotype has been observed in the same epidemic in Bangkok, nine showing 72.5 per cent to 91.4 per cent oligonucleotide homology whereas one produced a distinctly different fingerprint with from 55.7 per cent to 58 per cent homology.

This method offers highly sensitive and reproducible technical results. Generally, isolates from the same geographical area will be similar to each other, but differ from those of other areas. The fingerprint technique is a useful tool with which to investigate DEN epidemiology.

Synthetic RNA hybridization probes, complementary in nucleotide sequence to common and unique RNAase T1 oligonucleotides, have been constructed. Detection of DEN-2 topotype RNA by hybridization has been shown to be rapid and reproducible as an alternative to the laborious T1 oligonucleotide fingerprinting. Moreover, this technique allowed investigators to differentiate the African DEN-2 strains from the other topotypes and to point out variations between enzootic strains from West Africa and epidemic strains from East Africa.

The same classification of DEN-2 topotypes has been established by the technique of signature analysis using type specific monoclonal antibodies against the E protein.

More recently the nucleotide sequences of E proteins of several DEN-2 isolates have been determined by sequencing the viral RNA using synthetic oligonucleotide primers. Two DEN-2 strains have also been fully sequenced. The nucleotide divergence ranges from 1.5 per cent to 10.2 per cent difference whereas the variability in protein sequence ranges from one per cent to four per cent. Jamaican isolates of DEN-2 virus were shown to be closely related to strains from Vietnam by sequencing the junction between E and NS1. This suggests that the newly recognized (since the early eighties) topotype of DEN-2 virus in the Caribbean might have its origin in this part of South-East Asia.

These powerful analyses have not provided any molecular markers that correlate with disease severity. This problem, however, needs further investigation.

6. EXPERIMENTAL AND LABORATORY ANIMALS

In the laboratory, DEN viruses have been adapted to several experimental hosts, including both vertebrates and invertebrates, as follows.

6.1 Mammalian Hosts

Monkeys

Monkeys are susceptible to DEN viruses. Inapparent infection with DEN virus occurs in Cynomolgus fascicularis, Cercopithecus callitrichus, Macacus fasciatus, Macacus philippinensis and Macacus rhesus. Other species, Cynomolgus sinicus, Cercocebus aethiopis, and Pongo abun have been reported to be infected by injections of patient's blood. It was calculated that 9.5 mosquito infectious doses 50 (MID50) of DEN-2 and 22 MID50 of DEN-3 viruses were required to infect 50 per cent of the rhesus monkeys. Generally, infected monkeys show viraemia five to eight days after inoculation and viruses disappear from the blood 12 days later. Clinical signs such as hyperpyrexia and leucopenia are observable,
though only to a slight degree. An antibody response is evident in the recovery phase. In some monkeys killed after showing recognizable symptoms, myocarditis or nephritis was revealed to a slight degree. By intracerebral injection, a mouse-adapted DEN virus strain causes paralytic illness which resembles poliomyelitis clinically and histopathologically.

An inapparent infection is induced in chimpanzees (*Pan troglodytes*). Single strains of DEN virus types 1 to 4 at low passage levels after isolation in Thailand, New Caledonia or the Philippine Islands produced no overt, febrile or haematologically detectable subclinical disease in chimpanzees inoculated subcutaneously or intradermally. After primary inoculation, viraemia usually occurred from day 2 or day 3 through to days 5-7. After secondary challenge with DEN-2 virus 26 months after primary infection with DEN-1, DEN-2, DEN-3 or DEN-4, viraemia lasting 2-4 days occurred during days 3-6 in chimpanzees originally infected with DEN-1 or DEN-4, but not with DEN-2 or DEN-3. Homologous and heterologous neutralizing (N) antibodies were detectable in serum 4-6 weeks after primary infection of adult chimpanzees, but only homologous DEN-2 N antibodies were found in young chimpanzees.

Several species of New World monkey are also susceptible to DEN viruses and show evidence of viraemia or antibody response.

**Mice**

The mouse is the experimental host of choice. Several strains of DEN virus have been successfully adapted to mice, causing flaccid paralysis after intracerebral injection, and can be maintained by serial passage through the same route. In the early passages, a small but varying proportion of inoculated mice show clinical evidence of infection after an incubation period which varies from 5 to 35 days. The mice exhibit motor weakness and flaccid paralysis of one or more extremities with or without signs of encephalitis. Susceptibility to DEN viruses is variable among mice of different strains. In earlier studies, white mice about two weeks old were used. Suckling mice, one to three days old, are more susceptible to DEN viruses than are weaned ones. The suckling mouse is presently considered to be the universal host and best for inoculation without anaesthesia. After the first 24 hours, clinical signs including failure to eat, unusual colour, wasting or running, unusual activity, tremors or lying on the side, should be watched for. In general, signs of encephalitis usually develop within a few days. When the inoculated mice exhibit clinical signs, or immediately after death, DEN viruses can be found in the brain tissue. This method is routinely used for virus isolation in many laboratories, but will soon be replaced by other more rapid and sensitive methods. Infected mouse brain tissue can be used as a source for preparation of antigen in serological tests.

**Hamsters**

Two- to five-day-old hamsters have also been reported to be susceptible to intracerebral injection with mouse adapted strains of DEN-1 and DEN-2 viruses.

**Cave bats (*Myotis lucifugus*)**

The cave bat (*Myotis lucifugus*) was reported to be susceptible to DEN viruses (Hawaii mouse adapted strains), by intracerebral injection, for at least six passages in the laboratory.

**Other animals**

Tests for inapparent infections in dogs, rabbits, young hogs and guinea pigs have been negative. Epidemiological surveys conducted by Hammond et al. used sera collected from various animal species in South-East Asia. Anti-DEN complement-fixing antibody could not be demonstrated in sera obtained from horses, cattle, pigs, dogs, buffalo or cats.

**6.2 Embryonated Eggs**

Cultivation of DEN virus on the chorio-allantoic membrane of the developing chick embryo was
reported by a group of investigators without any substantial data. Sustained growth through repeated egg-to-egg passage, was, however, successfully conducted using high mouse-passages of an adapted Hawaii strain of DEN virus. A series of passages, initiated with virus of 10^1 mouse-passage transfers, was carried out through at least 48 egg-to-egg transfers without loss in titre, or changes in mouse pathogenicity or serological specificity.

6.3 Arthropod Hosts

*Aedes albopictus*

Replication of four prototypes of DEN virus has been demonstrated in both sexes of *Aedes albopictus* adult mosquitoes infected via the intrathoracic route for males and orally for females. DEN antigen can be detected by a direct fluorescent antibody technique in head smears of either male or female mosquitoes infected with any of the four DEN virus serotypes. Replication gives rise to a titre of 10^7 very rapidly. In males, infected by the intrathoracic route, the virus titre reaches its highest level earlier than in orally infected females. However, the titre attained in males is about five-fold lower than in females.

*Toxorhynchites* mosquitoes

Two- or three-day old laboratory-reared adult *Toxorhynchites splendens* can be infected by the intracerebral or intrathoracic routes using 0.17 microlitres per mosquito. Intracerebral inoculation is carried out conveniently through the dorsal part of the head capsule which lies between the neck and vertex and is called the occiput. Specific DEN antigen is detected earlier following intracerebral injection than it is following thoracic injection. The method offers an alternative technique for DEN virus isolation with more sensitivity than the conventional mouse inoculation method. The disadvantage of the adult *Toxorhynchites* inoculation method is the higher mortality rate of inoculated adult mosquitoes than injected larvae.

In a comparative study, susceptibility of *Toxorhynchites ambomensis, Toxorhynchites brevipalpis, Toxorhynchites rutilus* and *Toxorhynchites splendens* to parenteral infection with the DEN virus serotypes was shown to be equal, while *Toxorhynchites theobaldi* was relatively resistant. The intensity of immunofluorescence in the head squeeze was slightly less in *Toxorhynchites brevipalpis* infected with DEN viruses.

*Toxorhynchites larva inoculation*

A more sensitive and rapid method for DEN virus propagation has been developed using *Toxorhynchites splendens* fourth instar larvae. The larva is immobilized in ice and put on a moist gauze on the stage of a stereoscopic microscope. Inoculation is carried out through the dorsal epimere of the head capsule, in the general area behind the eye and between the frontal ecdysial line. Infected larvae are decapitated and smears are prepared by squeezing the contents of the head onto glass slides which are then fixed in cold acetone for ten minutes at 0-4°C. The fixed smears are either subjected to immediate staining or to storage at -70°C. By this method, DEN-1 and DEN-2 prototype viruses have been detected by the second day after inoculation. The overall advantages of this technique, compared to those using adult mosquitoes, are earlier detection, more intense fluorescence, higher survival rate of the injected insect and cleaner debris-free smears, thereby reducing the possibility of non-specific staining and facilitating interpretation.

Other mosquito species

In a comparative susceptibility study of 34 strains of Asian and Pacific mosquitoes infected orally with all four serotypes of DEN virus, several species of common man-biting *Aedes* were much more susceptible to oral infection with each of the four serotypes than was *Aedes aegypti*. These species include *Aedes albopictus* and members of the scutellaris group of the subgroup *Stegomyia* found in the South Pacific Islands.

Almost all species of *Aedes* tested were uniformly susceptible to parenteral infection with DEN viruses, with the exception of the species *tripertoides*. Species of all other genera were comparatively
resistant to this mode of infection. DEN viruses usually replicated to about the same extent in parenterally infected mosquitoes as they did in orally infected mosquitoes of the same species.

7. MOSQUITO TRANSMISSION STUDIES

7.1 Aedes Mosquitoes

Transmission of DEN-1 virus was determined in three strains of Aedes trisarius after oral infection. Rates of infection were similar to those observed in a control strain of Aedes aegypti. Three additional species belonging to the subgenus Protomachilea (Aedes brelandi, Aedes hendersoni and Aedes Zoosophus) were also susceptible to oral infection with DEN-1 virus but transmission could not be demonstrated, even though virus was detected in the salivary glands of the infected mosquitoes. Virus transmission was demonstrated for Aedes hendersoni following parenteral infection. Non-stegomyia mosquitoes may become involved in the transmission of DEN viruses to humans.

Colonized Aedes (Stegomyia) katherinensis mosquitoes from Australia were infected with the PR159 strain of DEN-2 virus using a membrane feeding technique and by intrathoracic inoculation. Analysis by indirect immunofluorescence revealed a 100 per cent infection rate of inoculated mosquitoes as compared to 45 per cent for orally infected mosquitoes. Few of the orally infected mosquitoes showed many viral antigens in the head and no virus transmission was detected. It is unlikely that Aedes katherinensis is of importance as a vector for DEN-2 in Australia.

7.2 Transovarial Transmission

Several studies concerning the transovarial transmission of DEN viruses have been conducted both in the field and in the laboratory. Even though the results of these studies are controversial, it can be concluded that transovarial transmission is not an important route.

Field studies carried out in Yangon, Myanmar (formerly known as Rangoon, Burma), during 1978 suggested that DEN-2 virus was transmitted transovarially by A. aegypti. In addition, laboratory studies suggested that DEN-1a virus was transmitted by the same route in one of five strains of A. aegypti. However, the filial rate was only 1/1543, even when all parent mosquitoes were infected. Moreover, all four DEN virus serotypes were transovarially transmitted by A. albopictus with a similarly low filial infection rate. This same study failed to demonstrate any evidence for transovarial transmission of DEN-2, DEN-3 and DEN-4 viruses by the different strains of A. aegypti. Another report revealed that DEN-2 virus could be transmitted transovarially by four different strains of experimentally infected A. aegypti, but the filial infection rates were also low, ranging from 0.3 to 1.2 per cent.

An extensive study was carried out in urban and rural areas of Thailand in the search for transovarial transmission of DEN viruses by A. aegypti and A. albopictus. DEN viruses were not isolated from 5766 larvae, 39 pupae, or 85 male A. aegypti collected from 35 houses in Bangkok in which one or more persons had had recent DEN virus infection. However, DEN-2 viruses were isolated from 14 of 268 female A. aegypti collected in 8 of the 35 houses. Virus was not detected in suspensions prepared from 73 A. aegypti larvae originating from eggs laid by three field-collected DEN-2 virus infected mosquitoes. Neither were DEN viruses isolated from 1459 male and 1740 female A. aegypti reared from immature mosquitoes collected in a rural village in which DEN virus was endemic.

8. IN VITRO CULTIVATION OF DENGUE VIRUSES

DEN viruses have been successfully propagated in several mammalian and arthropod tissue culture systems with or without cytopathic effect. Mouse adapted strains of DEN virus, DEN-1 (Mochizuki and Hawaiian strains) and DEN-2 (New Guinea C strain), multiplied with CPE in cultures of rhesus monkey kidney cells. Human lung, lymph node,
spleen, and thyroid and guinea-pig, porcine and hamster kidney cells have also been shown to support the growth of DEN virus.

Propagation in other established cell lines has also been documented, for example in LLC/MK2, BHK-21, and BSC-1 cell lines. Arthropod cell lines which are generally used for DEN virus, either for propagation or virus isolation, are the A. albopictus cell line of Singh (clone C6/36 of Igarashi) and the LSTM-AP-61 cell line (derived from Aedes pseudoscuerllaris). Using the C6/36 cell line, virus titres may reach a peak of 10⁸ PFU/ml in 72 to 96 hours.

The so-called "persistent infection" or "virus carrier state" is noted in certain culture systems infected with DEN virus. For example, prolonged maintenance of DEN-1 virus was noticed in experiments using monkey testicular cell culture, a human skin cell line (HuS 2806 strain), KB cells, African green monkey kidney cells and A. albopictus (Singh) cells. In these cases, active viruses are continuously detectable in culture fluid for an indefinite period of time.

9. SEROLOGICAL DIAGNOSIS

9.1 The Haemagglutination Inhibition Test

The Haemagglutination Inhibition test (HI) test has been most widely used to confirm several viral infections. The recommended procedure for dengue virus infection is that of Clarke and Casala adapted to the microtitration system, which has been described elsewhere. The patient's sera should be treated with kaolin or with acetone to remove non-specific inhibitors and then absorbed with goose red cells to eliminate the non-specific agglutinators. Haemagglutinating antigen is a 20 per cent suspension of crude infected suckling mouse brain in borate saline at pH 6.4, from which gross debris has been removed by centrifugation for 30 minutes at 3000 rpm. It is stable for four to six months and has been proved satisfactory. Ether-acetone or sucrose acetone extract of infected suckling mouse brain tissues gives more satisfactory results. Paired sera should initially be tested in the same run, using four to eight haemagglutinating units of a single broadly reactive antigen (usually DEN-1 or DEN-2 virus). If the paired sera have no antibody or do not show a significant antibody rise, both specimens should be re-tested against all four DEN serotypes. Known positive and negative sera should be included in each test to standardize the results and maintain laboratory quality control. Where chikungunya virus is known to be endemic, all paired sera should be tested against this antigen. On the other hand, where chikungunya is not known to be present, only a small proportion of the sera need to be tested against this antigen. Japanese encephalitis virus antigen may be included in the same run.

Finger-tip blood, collected onto filter paper and saturated through to the reverse side before being allowed to dry at room temperature, may be used with satisfactory results.

Interpretation of the test

Identification of infecting virus by serological testing is often difficult in persons who have had previous infections with other viruses of the same serological group as DEN viruses. High titres of antibodies can be found to certain antigens from several viruses in the group. In instances where sequential infection consists of a non-DEN flavivirus and a DEN virus, the IgM serum fraction may contain specific antibody only to the second infecting virus.

Primary antibody response

The primary antibody response to DEN infection is characterized by slow evolution of HI antibody which is often relatively monotypic, the absence of CF antibody until at least two weeks after the onset of illness, and monospecific neutralizing antibody. Definitive characterization of the primary response is established by demonstrating rising titres of anti-DEN IgM.

In practice, the DEN HI antibody titre is generally less than 1:20 in serum obtained before the fourth day after the onset of illness. There is
a four-fold or greater increase in titre in specimens obtained from convalescent patients (one to four weeks after onset of illness), with antibody titre not greater than 1:1280.

Secondary antibody response
The secondary antibody response is characterized by rapid evolution of HI and CF antibodies. All antibodies are broadly reactive. Definitive characterization of a secondary response is established by demonstrating rising titres of anti-DEN IgG.

Evidence of recent infection
In practice, HI antibody to DEN antigen(s) is less than 1:20 in serum obtained before the fifth day of illness and is equal to or higher than 1:2560 in serum from convalescent patients, or HI antibody is at least 1:20 in serum obtained before the fifth day after onset of illness, rising to at least 1:2560 in convalescent serum.

Presumptive recent infection
HI antibody is 1:1280 or greater in specimens from patients with acute illness with no four-fold or greater antibody rise in specimens from convalescent patients.

The Interpretation of DEN antibodies may be summarized as follows:

<table>
<thead>
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<th>First specimen</th>
<th>Second specimen</th>
<th>Interpretation</th>
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<tr>
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<td>After 1 - 4 weeks</td>
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</tr>
<tr>
<td>&gt; 4x and</td>
<td>&gt; 4x and</td>
<td></td>
</tr>
<tr>
<td>&lt; 1:2560</td>
<td>&lt; 1:2560</td>
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</tr>
</tbody>
</table>

*These criteria were developed from extensive experience with virologically and immunologically studied patients with DEN infections at the Department of Virology, US Army Medical Component, Armed Forces Research Institute of the Medical Sciences (AFRIMS), Bangkok, Thailand. If the sensitivity of the HI test systems in individual laboratories is standardized to that of WHO Laboratories, the use of recommended criteria should correctly classify the majority of DEN infections.

9.2 Neutralization Test
A variety of neutralization tests have been developed to measure dengue antibody. The 50 per cent end point plaque reduction neutralization test (PRNT) in LLC-MK2 cells has been widely used and is well described in the literature.

In brief, equal amounts of diluted serum and a suspension of DEN-1, DEN-2, DEN-3 and DEN-4 viruses containing 15 to 25 plaque-forming units/25 microlitres are incubated for 60 minutes at 37°C. All serum specimens are assayed in triplicate. The mean plaque count is then calculated. Following primary DEN virus infections, serum from patients with acute illness causes less than 70 per cent plaque reduction in all four serotypes, and the second serum shows monotypic antibody. With secondary infections, the first serum shows monotypic antibody and the second serum has antibodies to two, three or, most frequently, to all four serotypes. If the first serum already has antibody to two or three DEN viruses and the second serum reacts to three or four serotypes, it should be interpreted as a tertiary infection.

The PRNT test may be modified and performed as a semi-micromethod on BHK-21 cell cultures. This simplified method is favourable in terms of sensitivity in detecting DEN antibody (96 per cent), specificity at a screening dilution (95 per cent) and ability to detect seroconversion to three serotypes of DEN virus (93 per cent). Disagreements between the BHK-21 test and the LLC-MK2 test have been attributed to the greater sensitivity of the BHK-21 test in detecting DEN-2 antibody in acute phase sera and to apparent low levels of DEN-1/DEN-3 cross reaction in the same sera in all these tests. The BHK-21 test is much easier, faster and more economical than either of the LLC-MK2 tests.

Blood specimens, prepared from whole blood, and plasma or sera collected on filter paper strips, are satisfactorily used for the PRNT microneutralization test. Whole blood from a dried paper strip is eluted in phosphate buffered saline with antibiotics and kept overnight at 4°C. DEN antibody obtained by this method of specimen
collection is comparable to that obtained by other methods of collection from the same persons.

9.3 Complement Fixation Test

The complement fixation test (CFT) may also be used in serological diagnosis wherever facilities for this test exist. However, blood taken on filter paper is unsuitable for this purpose because it is lysed.

The CFT is useful since anti-DEN IgG fixes complement with DEN antigen. The presence of CF antibody in early convalescent phase sera signifies the secondary type of immune response.

9.4 Haemolysis in Gel

Single radial-haemolysis has been widely used to assay antibody to influenza virus since 1975. The test is based on passive lysis of sensitized erythrocytes by antibody in the presence of complement. This method is simple and rapid and has been successfully standardized for use in anti-DEN antibody determination. Briefly, washed sheep red blood cells (SRBC) are sensitized at 4°C for ten minutes with HA antigen of DEN virus in the proportion of 20 HA units per ml of 10 per cent SRBC in PBS. After washing three times with PBS to eliminate excess HA antigen, sensitized SRBC are mixed with 1.5 per cent agarose gel at 50°C and complement (at a final concentration of 1 per cent of sensitized SRBC and 1:30 of the complement). Fifteen ml of the mixture is poured onto a glass slide (9 x 10.5 cm) and 2.5 mm diameter wells are made. Five microlitres of inactivated (at 56°C for 30 minutes) patient’s serum, without any further elimination of non-specific inhibitors, is added to each well and the slide is then incubated at 37°C for 17 hours. Antibody is quantified by measuring the size of the haemolytic zone in the gel surrounding the well to which the patient’s serum has been added. Sensitivity of the haemolysis in gel (HIG) method is comparable to that of the HI test. However, it shows somewhat more specificity than the HI test in terms of differentiating between DEN antibody and Japanese encephalitis virus (JE) antibody.

9.5 Haemadsorption Immunosorbent Technique

The haemadsorption immunosorbent technique (HIT) is used as a rapid means of laboratory diagnosis for the detection of dengue-specific IgM. The technique is an antibody-capture-erythrocytes immunoassay. There is no need for physical separation of IgM from IgG. The well of a U-bottom microtitration plate is coated with anti-human IgM and absorbs the IgM from human sera. Dengue specific IgM is then detected by adding dengue virus hemagglutinin and a small quantity of goose erythrocytes. Centrifugation at 65g for 10 minutes facilitates the reading. Dengue specific IgM positive sera show haemadsorption whereas a button of red cells in the bottom of the well indicates the absence of dengue specific IgM.

Using this method, IgM antibody specific to dengue virus is detected in 56 per cent of convalescent phase sera from primary dengue virus infections and in 80 per cent of sera from secondary type infections, with titres ranging from 1:80 to more than 1:80 000. No IgM antibody has been detected in any acute phase sera. The HIT assay can be used in the laboratory diagnosis of single serum specimens, from suspected dengue patients, which have been reported inconclusive by the HI test. There are several advantages of the test; for example, no false positives occur and crude dengue antigen can be used in the test procedure.

9.6 Anti-Dengue IgM ELISA Test

Immunoglobulin M (IgM) antibody titres to DEN viruses are successfully measured by the antibody-capture ELISA test. The test has been used to distinguish DEN virus infection from Japanese encephalitis virus (JEV) infection. A sample is considered positive for JEV infection when the IgM ELISA titre for JEV antigen is over 200 and is four-fold or higher than the titre for any type of DEN antigen. The specimen is confirmed as positive for DEN infection when the IgM ELISA titre for one of the four serotypes of DEN antigen is over 200 and is four-fold or higher than the JEV antigen titre.
9.7 Other Serological Tests

Several test systems have been developed to demonstrate anti-DEN antibodies with special emphasis on rapidity, simplicity and specificity. These tests, however, are used in a few laboratories only. The staphylococcal agglutination-inhibition reaction\(^9\), and agglutination of antibody-erythrocytes sensitized by DEN haemagglutinins\(^9\), are two examples of such tests.

10. VIRUS ISOLATION

10.1 Specimens for Virus Isolation\(^9\)

Clotted blood or heparinized blood collected early in the course of illness is recommended for virus isolation. Serum or plasma should be separated and used immediately or stored at -60°C, or at a lower temperature, until used. For shorter periods of storage, materials can be kept at +4 to +8°C for up to 24 hours. Washed buffy coat, if available shortly after collection, is also a suitable source for dengue virus isolation. Homogenized tissue, especially liver, spleen, lymph node or thymus gland, obtained from autopsy, as a 10-20 per cent W/V suspension in buffer with one per cent protein stabilizer and antibiotics, may also be used for isolation. When vector mosquitoes are used for isolation they should be used in pools of 50-70 females and homogenized at approximately 10 per cent W/V suspension.

Before inoculation into any virus recovery system, serum or plasma samples should be diluted with suitable diluents.

10.2 Methods for Virus Isolation

There are at least three methods used for DEN virus isolation, namely, suckling mouse inoculation, inoculation of cell cultures of either mammalian or insect origin and inoculation of adult or larval mosquitoes.

DEN viruses are among the most difficult flaviviruses to isolate and identify. In the past, the two commonly used methods for primary isolation were inoculation into newborn mice\(^4\) and inoculation into cell cultures derived from vertebrates\(^4\). These two methods have been of limited value in the recovery of DEN viruses. Wild strains of DEN viruses are not usually pathogenic for suckling mice and many do not produce cytopathic effects or plaque in vertebrate cell cultures in the early passages. Moreover, recovery and identification by these methods are laborious and time-consuming and require several passages to obtain satisfactory antigen titres. More sensitive methods for this purpose are the direct and delayed plaques techniques, as reported by Yuill et al in 1968, using continuous monkey cell lines (LLC-MK2) for all four serotypes of virus\(^9\).

For further identification, the plaque reduction neutralization test in the same cell system, as described by Russell and Nisalak, may be used\(^9\).

Later developments, as reported in 1974 by Rosen and Gubler, involved the use of mosquitoes for detection and propagation of DEN viruses. Parenteral inoculation, either intrathoracic or intracerebral, of *A. albopictus* mosquitoes is found to be a much more sensitive method for the detection of DEN viruses than the plaque method in LLC-MK2 cells. Male mosquitoes are as susceptible to infection as females and can be used without the safety precautions necessary for the latter. Inoculated mosquitoes are kept at 28°C for 14 days. All four types of DEN virus replicate to high titres in this system after the early passages. Rapid identification of DEN viruses is carried out using the immunofluorescent staining procedure. Complement fixing antigen may be produced in male *A. albopictus* mosquitoes infected with DEN viruses and can be used, in conjunction with the prototype immune mouse ascitic fluid, to identify the viruses as to serotype.

Thet Win\(^5\) reported a method for intracerebral inoculation of *Toxorhynchites splendens* mosquitoes in 1982. Two to four day old *Toxorhynchites splendens* mosquitoes are used instead of *A. albopictus* mosquitoes and are kept at 32°C in 80 per cent relative humidity while being fed on ten per cent sugar solution. The virus can be detected by a direct immunofluorescent antibody technique as early as day five. Intrathoracic inoculation results in detection of viral antigen later than intracerebral
inoculation (on day 10-14). The larger size of the mosquitoes and the shorter period before detection of DEN viruses makes this method more popular than the A. albopictus inoculation technique.

A more rapid and sensitive method for isolation of DEN viruses from clinical specimens or mosquito pools has been developed by Lam et al., using Toxorhynchites splendens larvae as the hosts for inoculation\(^{(59)}\). This method is now in use in many laboratories. The application of this method in the field needs further evaluation.

Monoclonal antibodies can be used to identify epitopes on dengue viruses\(^{(86,87)}\). Henchal et al. successfully produced monoclonal antibodies of various specificities i.e., flavivirus group specific, dengue complex specific, dengue subcomplex specific and dengue type specific\(^{(20)}\). These four types of specific monoclonal antibodies were used to identify dengue virus isolates from different geographical areas by immunofluorescence or the plaque reduction neutralization test\(^{(59)}\). A monoclonal radioimmunoassay was also developed for detection of dengue virus in infected cell culture fluids and blood samples from dengue patients\(^{(99)}\). Kaufman et al. established a panel of monoclonal antibodies against E glycoprotein of DEN-2 virus and found that some antibodies had neutralizing activity both in vitro as tested by the plaque reduction neutralization test, and in vivo as tested by virus challenge following intracerebral inoculation of mice. However, some monoclonal antibodies had only in vitro neutralizing antibody activity\(^{(100)}\). Dengue specific monoclonal antibodies are now widely used for rapid identification of dengue virus.

A DEN-2 cDNA of about two kilobases, encoding part of the NS4B and NS5 proteins, has been used as a radioactive probe to hybridize viral genetic material in inoculated Toxorhynchites mosquitoes or in C6/36 infected cells\(^{(100)}\). As few as 11 plaque forming units of virus were detected. This method has potential as a diagnostic tool for identifying and typing dengue viruses in clinical and field specimens.

The flow chart in Figure 2 shows methods that can be used for isolation of dengue virus.

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**Figure 2. Flow chart for dengue virus isolation and identification**

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References:

Dengue Viruses


50. Sabin AB, Schlesinger RW. Unpublished studies on dengue reported to office of Surgeon General, US Army 1944 (cited in Ref 7).


Chapter 8
Vector Ecology and Bionomics
by
C.P. Pant
L.S. Self

1. INTRODUCTION

*Aedes aegypti* (L.) is the principal vector of dengue and dengue haemorrhagic fever in the world. It is widely distributed in the South-East Asian, Western Pacific, African and American Regions between isotherms 10°C January\(^{(N)}\) and 10°C July\(^{(S)}\) (see Figure 1). In Africa and Central and South America, this vector has also been known as the principal vector of urban yellow fever.

*Aedes aegypti* and other vectors of dengue and dengue haemorrhagic fever belong to the genus *Aedes*, subgenus *Stegomyia*. Exceptions are *Aedes*...
niveus, which has been involved in the transmission of dengue in the jungles of Malaysia and Viet Nam and Aedes samoanus and Aedes fijiensis, suspected as vectors in Samoa and Fiji respectively. These are leaf-axil breeding species belonging to the Finlaya subgenus.

It is generally believed that the original home of A. aegypti was Africa, from where it spread in conjunction with humans travelling from one part of the world to another. A. aegypti, which first established itself in the port cities of Asia, has now moved inland in many countries, as in Malaysia and Thailand(1,2,3). Urbanization has been a major factor in the spread of the species. Furthermore, in recent years, cases of dengue have spread into rural areas of Thailand. Dengue haemorrhagic fever (DHF) cases are at a higher level than observed in the early 1960s, but cases are now reported from up-country and not exclusively from Bangkok. This phenomenon must have been repeated in many other countries. Any major disturbance in human ecology, especially in urban areas in recent years, has served to amplify populations of A. aegypti. Examples of such disturbances include rapid growth of cities, population explosions, a steady deterioration of environment and standards of sanitation, and increases in the number of water-retaining waste containers and debris. These phenomena have brought many human and mosquito populations into close proximity. From large urban areas, the mosquito and the disease have travelled to smaller urban and rural communities.

Aedes albopictus, the essentially Asian mosquito (see Figure 2), has also been recognized as an important vector of dengue virus(3,4,5,6,7,8,9,10), although generally, where epidemics of DHF have occurred, A. aegypti has invariably been present. A. albopictus has been associated with the form of dengue seen in jungle areas and rural villages(9). In recent years, outbreaks of DHF have occurred in rural parts of Indonesia where A. albopictus is the predominant species(10). This species was also suspected of causing an outbreak of type 2 dengue (DEN-2) fever in the Seychelles(11). Dengue outbreaks associated with A. albopictus also occurred in the Solomon Islands in 1982 and in Japan in 1942.

Other Stegomyia species, such as the A. scutellaris complex, may also have been involved in the transmission of dengue in the absence of A. aegypti, for example A. polynesiensis in the Cook Islands in 1976 and A. rotumae in Rotuma, Fiji, in 1975. In Vanuatu (previously known as the New Hebrides) A. hebrides and A. aobae were implicated in the transmission of dengue. Other implicated species have been A. cooki in Niue, and A. scutellaris and A. pseudoscutellaris in Papua New Guinea. Aedes hakanssoni has been incriminated as a dengue vector in Ponape.

Aedes niveus (Finlaya) was considered responsible for an outbreak of dengue in a forest area of Viet Nam(12).

In summary, in Asia, A. aegypti is considered to be the principal vector of dengue/DHF followed by A. albopictus, which assumes importance in some areas and, together with A. aegypti, can be involved in serious outbreaks of DHF. Aedes polynesiensis and A. scutellaris have been implicated as vectors in the Pacific islands. Some other
species of the subgenus *Stegomyia* have also been involved in the Pacific islands, and on two occasions *A. niveus* (*Finlaya*) has been implicated in localized forest areas.

2. **Aedes aegypti**

2.1 Ecology and Bionomcs

Eggs and oviposition

The eggs are approximately 1 mm long and pale white, turning to an intensely black colour within a short time. They are elongate/oval in shape and under the microscope appear somewhat cigar shaped, with one end rather thicker and more abruptly tapered than the other. Fertilized eggs are deposited singly on the moist walls of the containers and the embryo develops within two to five days. The eggs are capable of withstanding desiccation for weeks or months, and possibly much longer. Eventually, when flooding occurs (once or several times), the eggs hatch. Lowered oxygen tension in the flood water provides a stimulus to hatching. The ability to withstand desiccation enables *A. aegypti* to be transported over long distances in dried receptacles or containers. This phenomenon also hinders control because eggs from dried receptacles can introduce infestation when water is added, thus continuing to present difficulties for control operations. The specific habits of egg laying have been exploited for detecting the presence of *A. aegypti* in an area by using ovitraps and for devising control methods by using autocidal traps. Among eight test materials, including filter paper, aluminium plates, glass plates, aluminium foil, wooden strips, car tyres, cement plates and plastic plates, the most attractive surface is that of car tyres. Females prefer to lay eggs on a surface with a high degree of dark colour, roughness and water absorption. The highest percentage of *A. aegypti* in Thailand is usually found in containers made from cement and in car tyres. Based on the oviposition habits and the colour attraction of *A. aegypti* females for egg laying, an ovitrap or oviposition trap was developed and used as a surveillance device for detecting the presence of *A. aegypti* in low densities (Figure 3). The trap was very successfully used as a supplementary control measure in Singapore Airport.

Differential responses to oviposition site by feral and domestic populations of *A. aegypti* have been studied. Results indicate that variations in response act as selective mechanisms separating gene pools of *A. aegypti*. The studies involved the use of water and plant infusions to compare the fecundity of populations. The percentage of females ovipositing in the infusion was higher than that of females ovipositing in water, by three per cent for the domestic strain and by 66 per cent for the feral strain.

Larvae and pupae

Larvae hang almost vertically at the water surface and swim with a distinct looping movement. When disturbed they swim to the bottom of the container. The larvae feed on particles of organic matter present on the bottoms or sides of containers by pharyngeal filtration of minute particles using fan-like brushes. The larvae also browse on the bottoms or sides of the containers, detaching matter from the surface over which they are gliding.

*Aedes* larvae can be distinguished by the naked eye from most other genera. The siphon is shorter than in most other culicines (it is lacking in anophelines). After hatching from the egg, the larva undergoes three successive moults. The fourth ecdysis or pupation gives rise to the pupa, which does not feed but actively swims and floats.

Field investigations in Bangkok between August and November 1987 showed the duration of different larval instars to be 17-20 days, in contrast to laboratory data which show this duration to be about 10 days at 28°C. Factors governing the duration of larval development are temperature, food availability and larval density in the receptacles.
Larval habitats

The breeding places of *A. aegypti* in Asia are usually located in or near houses in relatively clean water which is stored in containers and used for drinking and bathing. Metal drums or cisterns and ceramic or cement jars, both indoors and outdoors and used for water storage, as well as miscellaneous containers such as plastic pails, flower vases, flower pot plates, ant traps, discarded tins and bottles serve as breeding sites.

In Thailand and Indonesia, many studies have been carried out on the breeding habitats of *A. aegypti*. The total number of water containers per house in one of the suburbs of Bangkok was 9.0-14.0 (mean 11.0). Water jars, miscellaneous containers and ant traps were the most common habitats, and 44-gallon drums, cement baths, cement tanks and discarded tyres were also found to be common breeding sites\(^{21}\). In urban Jakarta, immature stages were found in or near houses in containers with relatively clean water used for drinking or bathing. An average of 185 containers was found per 100 houses, of which 60 were positive for *Aedes* immatures. The mean potential water storage capacity per house was 17.3 litres. Water jars were the most common containers found but "Bak Mandi" (cuboidal or oblong concrete reservoirs) held more water\(^{22}\). In Singapore, natural and domestic habitats, including rubber tyres, building equipment, machinery parts and boats are the common breeding habitats for *Aedes spp*\(^{23}\). To give the complete list and importance of these habitats, a table from the latter publication\(^{23}\) has been reproduced (Table 1), in which *A. aegypti* and *A. albopictus* have been grouped together.

The relative importance of these habitats may vary from country to country. As stated earlier, *A. aegypti* is an introduced species in Asian cities whereas *A. albopictus* is the native species. *Aedes albopictus* has both a natural and suburban distribution where rich vegetation, high altitude or poor accessibility and communications exist. Some observations have shown *A. aegypti* to be replacing *A. albopictus*, although the observations made and methods used have been inconclusive\(^{23}\). Sharing of habitats is uncommon in both urban and rural areas, however *A. aegypti* takes a slightly shorter time to complete its development from egg hatching to adult emergence. It is probable that the pattern of distribution of the two species is not the result of competitive displacement, but may result from factors that favour the rapid increase of *A. aegypti* populations. In Bantul (Central Java), a densely populated area, outbreaks of DHF have occurred in recent years and larval habitats of *A. aegypti* and *A. albopictus* have been studied\(^{10}\). It has been found that both species are common in the villages,

### Table 1. Number of different types of breeding habitat of *A. aegypti* or *A. albopictus* in Singapore City\(^{23}\)

<table>
<thead>
<tr>
<th>Type of habitat</th>
<th>Number</th>
<th>Percentage of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural habitats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earth drains, pits, branches</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Coconut shells</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Vehicle tracks</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Lead pits</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Molussc shells</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ground pools</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Domestic habitats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant traps</td>
<td>2050</td>
<td></td>
</tr>
<tr>
<td>Earthenware jars</td>
<td>1533</td>
<td></td>
</tr>
<tr>
<td>Bowls</td>
<td>830</td>
<td></td>
</tr>
<tr>
<td>Tin cans</td>
<td>705</td>
<td></td>
</tr>
<tr>
<td>Tanks</td>
<td>813</td>
<td></td>
</tr>
<tr>
<td>Drums</td>
<td>457</td>
<td></td>
</tr>
<tr>
<td>Pots</td>
<td>299</td>
<td></td>
</tr>
<tr>
<td>Bottles</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Plates</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>Vases</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>Basins</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Pails</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Cupes</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Barrels</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Pans</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Dishes</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Others (total of 13 types)</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Rubber tyres</td>
<td>291</td>
<td>3.5</td>
</tr>
<tr>
<td>Water and sewage disposal installations:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drains</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Others(^a)</td>
<td>29</td>
<td>0.9</td>
</tr>
<tr>
<td>Building equipment and machinery parts(^b)</td>
<td>29</td>
<td>0.4</td>
</tr>
<tr>
<td>Boats</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Water-pipes, manholes, sewage tanks, stop-cock pits, sumps, gullies, etc.
\(^b\)Cement mixers, concrete blocks, scrap iron, etc.
but *A. aegypti* is found primarily in "Bak Mandis" indoors and outdoors, and also in clay pots. *Aedes albopictus*, on the other hand, is found primarily in cut bamboo stumps outdoors and also in tree holes, coconut shells, clay pots and "Bak Mandis". There is considerable overlap in the breeding habitats of the two species in Bantu!

In Pondicherry, a town in South India, a survey was carried out in 2580 coconut tree holes during the rainy season and eight tree holes during the dry season. *Aedes aegypti* and *A. albopictus* were found in all months \(^2\). In Pune city (Maharashtra State in India), mud, brick, cement or stone containers were used most frequently by *A. aegypti* as breeding sites, followed by metal containers, rubber tyres and other receptacles \(^3\). Although *A. aegypti* is mainly a container-breeding mosquito in Asia, in a few villages on an island near Cuddalore in S. India it has been found breeding in wells. These wells are shallow, as the water table in these parts is high.

The reasons for water storage vary in different regions. In some areas of Thailand people prefer rain water for drinking, particularly on the coast where the sub-soil water may be salty. In addition, where a piped water supply is not available and labour is involved in drawing water, people prefer to store water. Even when piped water is available, disruptions in the supply may lead to the practice of extensive water storage. In forest areas there may be a lack of water, especially during the summer. In Thailand, most of the houses are wooden and, since people are afraid of fire outbreaks, they prefer to have copious quantities of stored water available. Water storage practices assume cultural patterns in warm and humid climates where frequent bathing and washing of clothes is necessary.

Ecology, seasonal history and life budget

From May 1973 until June 1974 the adult and immature populations of *A. aegypti* were monitored in four districts of urban Jakarta. There was no consistent seasonal pattern of population fluctuation of either adults or immatures. Although there was a clear seasonal pattern of rainfall, most breeding occurred indoors, thereby minimizing the effect of increase of water filled containers outdoors during the rainy season \(^4\). The magnitude of seasonal changes in larval populations of *A. aegypti* was studied in Bangkok, Thailand. There was some reduction in larval populations during the cool and hot seasons and the magnitude and timing of the fall varied from place to place. The reduction was in the order of 11-20 per cent \(^5\). An adult population of *A. aegypti* was studied in the residential compound of a Buddhist temple, Wat Samphaya in Bangkok, using mark-release and recapture techniques. The 24-hour survival rate using mathematical models was 0.81 for females, and no significant differences were found between different months. There was no striking increase in population during the wet season and the study indicated that fluctuations in the amount of movement, expectation of life and population size through the year are inadequate to account for the changes in the incidence of dengue haemorrhagic fever \(^6\).

In Singapore, where the suitability of most habitats of *A. aegypti* and *A. albopictus* depends on rain, fluctuations in the populations were found to be associated with rainfall except during the middle of the year, when there was no increase in rainfall at the time of the peak in population. It has been suggested that other population regulating factors in addition to rainfall also determine fluctuation in populations of *A. aegypti*. *Aedes albopictus* population fluctuation has been shown to bear a close relationship to rainfall \(^7\).

A study made on the daily emergence of *A. aegypti* in Sonapat, India, showed that high emergence (up to eight-fold in June as compared to February) was associated with extremes of climate. No clear increase in the emergence pattern occurred during the rainy season \(^8\).

Studies on the life budget of *A. aegypti* have also been carried out in Bangkok, Thailand. Variation in the number of emerging adults was found to depend on changes in the mortality of the immature stages rather than on the variation in the number of eggs laid. Both early and late larval instar mortalities were important, the former being
more significant during March-August. The mortality occurring between the egg stage and second instar larval stage was density dependent. There was no close trend of association between mortality and season except for a fall in larval mortality between April/May, preceding the annual increase in incidence of haemorrhagic fever, which usually occurs in June\(^{20}\).

The adult mosquito

The adult mosquito, after emergence, rests on the walls of the breeding site for a few hours to allow the exoskeleton and wings to harden. Approximately 24 hours after emergence both sexes can mate and females can take a blood meal. These two activities often take place simultaneously since both males and females are attracted towards vertebrate hosts. Wing beat frequency of the unfed females and the sound generated may also be attractive to the males. Generally, once inseminated, the females do not mate again.

The biting habits of mosquitoes have been used not only to estimate mosquito population densities but also to determine the possible relationship of these densities to epidemics of mosquito-borne diseases.

*Aedes aegypti* is highly anthropophilic in Asia. The diurnal periodicity of attraction of *A. aegypti* to human bait was studied in Jakarta\(^{31}\). Although biting seems to occur throughout the whole day, there are two distinct peak periods of diurnal activity of females, which are similar throughout the year. The mid-morning peak occurs between 0800 and 1300 hours and the mid-afternoon peak between 1500 and 1700 hours. The mean parous rate for females collected in the area is higher during the rainy season than in the dry season. In a study in Bangkok, it was shown that during the month of January, in the middle of the cool-dry season, a statistically significant reduction in the biting rate occurs (1.48 as compared to 4.77 in September)\(^{32}\). This finding is somewhat at variance with earlier studies which found that the absolute populations of mosquitoes did not fluctuate seasonally. Studies were therefore carried out to ascertain the feeding frequencies and gonotrophic cycle of *A. aegypti* in Bangkok throughout the year\(^{35}\). During the cool-dry season, a delay in feeding for the first time, of one to two days, was found to occur, thus reducing the feeding rate.

The above may explain the fact that, although cases of DHF are found in Thailand throughout the year, there is a pronounced seasonal change. Recently, the efficiency of *A. aegypti* as the vector of Dengue virus 2 (DEN-2) was studied in Bangkok, and it was shown that minor changes in temperature greatly influence the efficiency of transmission\(^{34}\). DHF cases in Bangkok follow an annual cycle, with peak rates in the mid-rainy and cool-dry seasons. Rising case rates are observed during the hot season. The pattern remains the same, but the magnitude may vary between the mild and severe years by a factor of ten. The total number of cases is strongly correlated with the number of cases in March and May. Linear regression analysis of monthly DHF case rates shows that high DHF case rates can be correlated with high environmental temperatures during the cool season. Although the mechanism of these events has not been studied, it is known that the gonotrophic cycle, feeding frequency, and the incubation period of the virus in the mosquito are affected by cool season temperatures.

2. VECTOR COMPETENCE AND VIRUS STRAINS

Recent work has demonstrated that, among the factors influencing the distribution and spread of epidemic dengue haemorrhagic fever, the virus serotype and strain and vector competence may be important in determining the magnitude of the epidemic and the amount of severe disease. In Tahiti and New Caledonia, for example, in 1971-72, in Tonga in 1974, and in Fiji in 1974, differences in virulence of both DEN-1 and DEN-2 were observed. In Indonesia, Dengue 3 (DEN-3) seems to be associated with explosive epidemics and severe disease\(^{35}\).

The comparative susceptibility of different strains of *A. aegypti* and *A. albopictus* also seems
to vary with the geographical location. Most strains of *A. aegypti* from South-East Asia and the Pacific show a higher susceptibility to DEN types -2 and -3. Low susceptibility of *A. aegypti* to oral infection with dengue viruses seems to be associated with milder outbreaks. However, caution has to be exercised in interpreting such data.  

3. **Aedes albopictus**

*Aedes albopictus* is widely distributed in Asia. Recently it has also been recorded in the State of Texas, United States of America, where it is suspected of having been introduced through international cargo and shipping. In parts of its range, this species is a vector of dengue and DHF, generally in conjunction with *A. aegypti* (see Figure 2 for distribution).

The introduction of *A. albopictus* into the Solomon Islands in 1978 had important public health consequences because a dengue fever outbreak occurred in Honiara in 1982. There is concern that this species may continue to spread into the South and Central Pacific regions, and it is now known that it has extended its range to at least Truk Island, Majuro and Ponape in the Trust Territory of the Pacific Islands.

*Aedes albopictus* is primarily a species found in forested areas and where there is natural vegetation. The species has also now adapted to urban environments. *Aedes albopictus* is also prevalent in more northern latitudes than is *A. aegypti*.

Adults of *A. albopictus* are easily distinguished from those of *A. aegypti* by distinct markings on the dorsum of the mesonotum.

3.1 Larvae, Pupae, Larval Biology and Habitats

Larvae can be easily distinguished, under a dissecting microscope, from those of *A. aegypti* by the different shape of the comb scales and pecten teeth in the terminal segments. *Aedes albopictus* also has four ventral brushes in contrast to the five which are found in *A. aegypti*. Larvae are similar in their swimming action and behaviour.

The distribution of the larger proportion of the two species is allopatric, with a zone of overlap in small gardens. *Aedes aegypti* inhabits domestic premises while *A. albopictus* remains outdoors. In some cities, where vegetation is abundant in some parts as opposed to human habitation in other parts, *A. albopictus* abounds in the former and *A. aegypti* in the latter sections. In Indonesia, in some villages where both species have been recorded, *A. aegypti* is primarily found in the "Bak Mandis" indoors and outdoors, whereas *A. albopictus* is found in cut bamboo stumps, tree holes, coconut shells, clay pots, etc. (see also *A. aegypti* section, in this chapter). In both Saipan, in the Northern Marinas, and Guam, *A. albopictus* is widespread and has replaced *A. aegypti*.

3.2 Ecology and Seasonal History

The density of *A. albopictus*, due to the breeding habits of the species, is more influenced by rainfall than is the density of *A. aegypti*. In Indonesia, a close correlation occurs between the beginning of the rains in October and the onset of the dengue epidemic a few weeks later. In Singapore, where the existence of most of the habitats of *A. albopictus* depends on rain, fluctuations in the population are associated with rainfall.

The eggs of *A. albopictus*, as also those of other *Stegomyia*, can withstand long periods of dryness. The duration of the life cycle, from oviposition to emergence of adults, may range from slightly over one week to six or seven weeks. It has been suggested that *A. albopictus* has a lower fecundity and longer life cycle than *A. aegypti*, thus giving the latter species a biological advantage.

The adults of *A. albopictus* are generally found biting outdoors and, like *A. aegypti*, exhibit two peaks of biting activity, in the early morning and late afternoon. In domestic and peri-domestic environments the species feeds readily on man, but in forests and rural areas it feeds on other hosts.

The principal resting places of *A. albopictus* are outdoors, presumably in vegetation, and only small numbers have been collected indoors.
4. **Aedes polynesiensis**

*Aedes polynesiensis*, a member of the *scutellaris* group, is the well-known vector of sub-periodic bancroftian filariasis in several islands of the South Pacific and has also been known to transmit dengue virus. The species has been found in American Samoa, the Cook Islands, Fiji, Western Samoa, and Tahiti and the Marquesas Islands in French Polynesia. Epidemiological evidence indicates that dengue outbreaks in Tahiti and American Samoa were caused by *A. polynesiensis*. *Aedes aegypti* has generally been present in these areas and has been shown to be more competent as a vector than *A. polynesiensis*, and thus this latter species can be considered to be a secondary vector in many areas.

4.1 Breeding Habitats

*Aedes polynesiensis* shows great plasticity in the selection of its breeding places. It breeds in plant containers, artificial containers and crab holes. In the Cook Islands it has been noted that the important breeding sites of *A. polynesiensis* are:

- Cisterns for collecting rain water
- Drums for storing water
- Coconut shells and discarded tins
- Ant guards
- Tree holes

In Fiji, crab holes seem to be important breeding sites of *A. polynesiensis*, the greatest number occurring within about a mile of the coast or tidal rivers. The species has been found breeding in the leaf axils of *Pandanus*, but this is not a typical breeding site and the occurrence is rare.

4.2 Ecology, Seasonal History and Bionomics

*Aedes polynesiensis* is a sylvatic and peridomestic species. The mosquito does not rest indoors, except on rare occasions, and may be found in shaded vegetation. The range of dispersal is believed to be about 100m. The species bites during the daytime, with early morning and late afternoon peaks. Man is the preferred host, although the species may feed on horses and dogs. Most biting occurs out of doors, but the female will readily go indoors to bite.

The mean duration of the immature stages is around eight to ten days under laboratory conditions (at 26-29°C). The larval stages tolerate salinity well.

In areas of seasonal rainfall, *A. polynesiensis* populations build up during the rainy season. Where the seasons are not well marked, as in Western Samoa, there are not well defined population changes. Where heavy breeding takes place in crab holes, such as in Fiji, adults may be abundant during relatively dry periods.

Under laboratory conditions at 27°C, the adults have been reported to survive for about 20-22 days on average.

5. Other Vectors

*Aedes pseudoscutellaris* occurs in Fiji and is very close to *A. polynesiensis* in morphology. It mostly breeds in tree holes, bamboo stumps and crab holes. It bites in the daytime.

*Aedes cooki* is the only species of the *scutellaris* group in the Niue Islands. It is a likely vector of dengue, although it is now known that *Aedes aegypti* also occurs in Niue. *A. cooki* breeds in rain-water containers, coconut shells and tins. It bites outdoors in the daytime.

*Aedes rotumae* is the only species of the *scutellaris* group on Rotuma Island of Fiji. It breeds in artificial water containers and coconut shells.

Among the other possible vectors are *A. hakanssoni* in the Caroline Islands, *A. hebrideus* and *A. aobae* in Vanuatu, and *A. tabu* and *A. tongae* in the Tonga Islands.

These are all outdoor biting species and breed in tree holes, artificial water containers, coconut shells and leaf axils.
6. METHODS OF SURVEY

Surveillance of vectors is an essential step in the planning of control measures and their evaluation, and in studies to determine the risk of outbreak of dengue/DHF. Surveys are also necessary for studying the ecology and distribution of vectors.

Surveys enable information concerning the presence of vectors, their frequency of occurrence, their abundance and distribution in time and space, their movements including migration, and their establishment in other areas to be obtained. These surveys also assist in stratification of areas where outbreaks of dengue fever can occur. Vector surveillance should be routine and include the monitoring of ecological and epidemiological parameters from both the virological and entomological points of view. The objectives of these surveys have been summarized as follows:

- to pinpoint high risk areas (areas with high vector density and high disease endemicity) through the plotting of vector distribution and DHF cases on maps, so that these areas can serve as priority areas for control during both normal and epidemic conditions;
- to detect, through routine surveillance, any changes in vector density, distribution, or other epidemiological parameters relating to the vectorial capacity of the vectors;
- to determine the seasonal population fluctuations of the vectors so that special emphasis can be given to the maintenance of control and alertness during peak periods, and
- to determine the major breeding places in domestic environments so that source reduction or elimination, with public participation, can be carried out through health education campaigns and law enforcement.

6.1 Larval Surveys

Due to the nature of the larval habitats and the ease with which larvae can be collected, larval surveys are commonly used for *Aedes* species, and involve the collection of larvae or pupae. The immature stages are collected from water-holding containers found both inside and outside houses. Information concerning the locality, date of survey, precise location and classification of the container or source is carefully recorded. Receptacles which are negative for immature stages on examination are also recorded. The exact format of such records will vary from place to place depending on the type of breeding site and the purpose of the survey. Larval surveys may be of three types:

- those concerned with all larvae, or a number of larvae, from all positive containers;
- those concerned with a single larva from each positive container, and
- Visual.

In order to carry out the above, which results in a saving of time for field workers, detailed information about the composition of the *Aedes* species breeding in the containers should be available. Visual larval surveys, and the one larva per container survey are only accurate when one species, e.g. *A. aegypti*, is found, as is the case in some urban areas. In the urban areas of Bangkok, Thailand, one larva per container surveys were found most suitable for comparison of *A. aegypti* populations between localities and seasons.

The method involving all larvae has been used in Singapore, where both *A. aegypti* and *A. albopictus* are common. Both methods have advantages and disadvantages and their use depends on the objectives of the survey.

**Larval indices**

The commonly used larval indices are as follows:

- **House or premises index**

This is the percentage of houses or premises with one or more habitats positive for *A. aegypti* or related species. It is calculated as follows:

\[
\text{House or premises index} = \frac{\text{No. of infested houses}}{\text{No. of inspected houses}} \times 100
\]

- **Container index**

Percentage of containers infested = \(\frac{\text{No. of infested containers}}{\text{No. of inspected containers}} \times 100\)
In examining the containers, only those which have water in them are counted.

Breteau index
Originally this index was used in connection with A. aegypti, and

\[ \text{Breteau index} = \frac{\text{No. of infested containers}}{\text{No. of inspected houses}} \times 100 \]

The above are the most commonly used indices, but several others have also been proposed. For example, in order to relate the indices to the risk of disease transmission, it is important to relate the number of infested containers to the human population, and the Stegomyia index has been proposed, as follows\(^{(38)}\):

\[ \text{Stegomyia index} = \frac{\text{No. of positive containers}}{\text{No. of people living in the area or premises surveyed}} \times 1000 \]

This index involves obtaining accurate information about the number of people living in the premises surveyed, which is often easier than obtaining an accurate demarcation of different householders in crowded urban communities, as is required in the calculation of House or Breteau indices. Thus, for urban areas the Stegomyia index has advantages. Where it is not possible to obtain accurate population figures, such as in some rural areas, there may be difficulties. The same may be true if the population is unstable and characterized by emigration or immigration.

From the epidemiological viewpoint, a more exact relationship between mosquito and human density is important and the following index has also been proposed\(^{(38)}\):

\[ \text{Stegomyia larval density index} = \frac{\text{No. of larvae in the area}}{\text{No. of humans in the area}} \times 1000 \]

Thus, in addition to accurate estimates of the human population, this index requires accurate estimates of the number of larvae in an area, which may involve considerable additional work.

The Breteau Index is generally considered the best of the commonly used indices (such as the House or premises Index and the Container Index) since it combines dwellings and containers and is more qualitative and of more epidemiological significance.

The House Index is most frequently used and understood. It also involves less labour because, when the first positive container is located in a house, there is no need to proceed further. This index does not take into account the number of positive containers in an infested house. The House Index gives an idea of the percentage of houses positive for vector breeding and hence the percentage of the population at risk. If the index is high, transmission occurs easily to neighbouring houses, and if the index is low transmission occurs less rapidly.

The Container Index, although not so useful from the epidemiological point of view, is a useful comparative figure, especially when evaluation of control measures is being carried out.

The House Index and Breteau Index have been shown in the West Indies to be highly correlated\(^{(38)}\). At low rates of infestation, the House and Breteau Indices are essentially the same. At higher infestation rates a divergence between the two rates has been noticed. The World Health Organization (WHO) developed a WHO Density Figure (on a scale of 1 to 9) which shows the following relationship between these indices\(^{(38)}\):

<table>
<thead>
<tr>
<th>Density figure</th>
<th>House index</th>
<th>Container index</th>
<th>Breteau index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-3</td>
<td>1-2</td>
<td>1-4</td>
</tr>
<tr>
<td>2</td>
<td>4-7</td>
<td>3-5</td>
<td>5-9</td>
</tr>
<tr>
<td>3</td>
<td>8-17</td>
<td>6-9</td>
<td>10-19</td>
</tr>
<tr>
<td>4</td>
<td>18-28</td>
<td>10-14</td>
<td>20-34</td>
</tr>
<tr>
<td>5</td>
<td>23-37</td>
<td>12-20</td>
<td>33-49</td>
</tr>
<tr>
<td>6</td>
<td>38-49</td>
<td>21-27</td>
<td>50-74</td>
</tr>
<tr>
<td>7</td>
<td>50-59</td>
<td>28-31</td>
<td>75-99</td>
</tr>
<tr>
<td>8</td>
<td>60-79</td>
<td>32-40</td>
<td>100-199</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
<td>41</td>
<td>200</td>
</tr>
</tbody>
</table>

All the above three indices are, however, estimates of frequency and not actual numbers. Hence they are empirical.
The adult and larval indices for *A. aegypti* in towns in Southern India have been studied and compared. In this study, adults and larvae were collected simultaneously in the same house, and the house indices for both were positively correlated. The adult indices were higher than the larval indices, except when densities were very low.

### 6.2 Adult Surveys

Since only adult mosquitoes are involved in disease transmission and control measures may be directed against adults, it is important to carry out surveys for adult mosquitoes as well. In such surveys the mosquitoes are collected by aspirator either while they are resting or when they are at bait. The dengue vectors are generally arthropophilic, hence animal baits are not used. The following adult indices are generally measured:

- **House density index.** The number of female mosquitoes per house, or the number of females per house per unit of time.
- **Biting rate index.** The number of female mosquitoes taken at bait per unit of time.
- **Net index.** The number of female mosquitoes caught per man hour of collection by net.

These indices are good for domestic or intradomiciliary species, especially the House Density Index. This is obtained by collecting all adult mosquitoes or collecting indoor resting mosquitoes during a unit of time, e.g. 15 minutes, using an aspirator (mouth or battery powered), torch light or hand sweep-nets. Adult *A. aegypti* are generally found resting on hanging objects, such as clothing, shady and dark corners on the wall, and furniture, in bedrooms and other rooms. The results are expressed as the number collected per man hour or the number collected per house. The mosquitoes are identified as to species and sex. Catching stations can be selected at random or, when specific or comparative studies are to be made, fixed catching stations can be employed. Too frequent collection can reduce the population and affect the purpose of the survey.

### Biting or landing catches

*Aedes* mosquitoes can also be collected on human baits. The worker can collect mosquitoes from his own body (exposed legs) or that of his helper. Generally 15 to 20 minutes are spent in each room and an aspirator is used to collect all mosquitoes landing or biting. On the basis of the peaks of activity of *Aedes*, the collections should be carried out between 09.00 and 11.00 hours. The collector(s) should sit in a dimly-lit room and expose the legs up to the knees, removing shoes and socks. Male mosquitoes are very often collected in this kind of catch, but they are not counted when calculating the biting or landing index.

In order to determine the best time to carry out these catches, it is appropriate to do some 12-14 hour catches, from before dawn until dusk. Should comparisons between the seasons or between pre- or post-spraying be required, all the conditions should be standardized.

### Net index

The adult net index can also be determined and, in this method, the collector utilizes a small sweeping net to collect the resting or flying mosquitoes from various resting sites, such as clothing, under furniture, dark corners, etc. The collector should spend a certain standardized period in each room, such as 15 to 20 minutes and, once standardized, this period should be used all the time.

### 6.3 Ovitrapt Collections

Black jar ovitraps have been used for surveillance of *A. aegypti* (see this chapter section 2.1). Local variations in the size of jar and type of paddle have been made, for example using velour paper or cloth as paddles. Care should be taken in selecting the paddle to ensure its capacity to absorb water. The paddle is placed inside the jar which is half filled with clean water, with the rough side of the paddle facing towards the middle of the jar (see Figure 3). Ovitraps are specially useful when the density of the mosquitoes is low, e.g. immediately after an adult control programme.
The sensitivity of the traps depends on their placement. The approach has been used for studying the distribution of A. aegypti and A. albopictus on the small islands of Penang. Traps should be placed on or near the ground away from the interference of small children and animals. When many egg-laying sites, such as water containers or tyres, are present, the efficiency of the traps is reduced. The traps should also be kept away from direct sunshine and should be sheltered from rain. Traps can be randomly distributed or organized as a trap-line or grid. Each jar and paddle should also carry an identification number.

Traps should be serviced weekly and the old paddle removed and replaced by a clean one. The old paddles should be placed in separate plastic bags, marked and carried to the laboratory. The water from the jars should also be poured into enamel bowls and the larvae counted and identified. Jars should be cleaned using a stiff brush prior to using again.

If species other than A. aegypti are present, it is necessary to rear the eggs from the paddles for identification purposes. If only A. aegypti is present in the area, the paddle should be air-dried and the eggs examined under the low power of a dissecting microscope.

The operation of ovitraps in port or airport areas for several weeks is a sensitive and accurate way of determining the presence of A. aegypti. When properly used, the ovitrap is a sensitive and inexpensive device for monitoring the Aedes population whilst utilizing less manpower than is required by larval and adult searches. The method is also less subject to human bias and errors of sampling.

The results can be expressed as:
- Average number of eggs of Aedes spp. per ovitrap.
- Per cent of positive ovitraps.

For quantitative information, it is essential that a large number of ovitraps are used. The number of traps used depends on the objectives of the survey and may range from 18-50 to a few hundred.

In some areas, such as that covered in a study in Jakarta, ovitraps do not provide good results. Therefore, prior to their use, some pre-testing of the method is required.

An interesting development of the use of this sampling device is its use in Aedes control (see section on Control of A. aegypti).

6.4 Longevity of the Vector

Since disease transmission rates very much depend on the longevity of the vector(s), some estimation of this helps in understanding the risks involved and the efficacy of control measures, if directed towards adults. Although there are no direct methods available for the determination of longevity of A. aegypti or other Stegomyia, approaches similar to those used for malaria vectors could be utilized, e.g., determining the proportion of parous females and the probability of survival through one day, according to the following consideration:

\[
\text{Probability of survival through one day} = \frac{N}{\sqrt{\text{proportion parous}}}
\]

where \(N\) = gonotrophic cycle in days.

The proportion of parous females can be determined by dissecting the females and examining the
ovaries under the microscope. When dissected in normal saline solutions, the presence or absence of one or more dilatations in the stalks of the ovarioles can be seen. Alternatively, the ovaries are allowed to dry on a slide, when the presence or absence of “skeins” of the tracheal system can be seen.

It was shown in Singapore that the trend of the disease followed the trend in parous rate of both vector species with a time lag of one to two months. Considering the increase in longevity of A. aegypti during the rainy season in Thailand and Jakarta and that there are no marked fluctuations in the A. aegypti population throughout the year, it appears that longevity may play a dominant role in outbreaks of DHF during the rainy season.

6.5 Vector Competence

Although vector competence studies may not be possible in routine surveys, they are indicated when studies on the epidemiological importance of different species are to be carried out.

It has been shown that A. aegypti and A. albopictus are both susceptible to oral infection with all four dengue serotypes and that there is no difference in the ability of the two vectors to transmit dengue. In fact, A. albopictus was shown to be equally as competent as A. aegypti in transmitting a Dengue-3 strain.

Aedes aegypti, A. polynesiensis and A. pseudoscutellaris in Fiji were found to be capable of transmitting Dengue 2, 3 and 4 in the laboratory, and thus were potential vectors. Aedes aegypti was, however, the most efficient.

7. VECTOR SURVEILLANCE AND EPIDEMIOLOGICAL INTERPRETATION

7.1 Vector Surveillance

The main objective of vector surveillance is to obtain information to assist entomologists and health planning authorities in the following:

- determining the major breeding sources of potential and actual vectors in domestic and peridomestic environments;
- stratification of areas according to density to allow pinpointing of high risk areas and areas with high Aedes densities, and plotting of vector distribution and DHF cases on maps to decide on priorities for control measures;
- determining seasonal fluctuations of the populations of vectors, to ensure alertness during peak periods;
- providing information for prevention of outbreaks of DHF by relating vector indices to other epidemiological information, so that action for control of the disease can be taken in time;
- determining any changes in vector density, distribution and/or vectorial capacity to ensure proper strategies for control; and
- determining susceptibility/resistance of vectors to insecticides.

The various indices which can be included in surveillance programmes have been discussed above. The exact selection of procedures will depend upon the specific objectives of the programme and the availability of manpower and resources.

Within its range of distribution, dengue haemorrhagic fever is mostly confined to densely populated cities and suburbs, with only sporadic cases in rural areas. However, in Indonesia in 1978, about 30 per cent of cases came from rural areas. Whereas A. aegypti is mostly associated with outbreaks of the disease, there is evidence from Myanmar (Lashio outbreak of 1975) and Indonesia that A. albopictus is important locally.

In the Western Pacific islands, species other than the main vector (A. aegypti) may be involved, and may be localized within certain islands only.

The methods of vector surveillance presently recommended by the WHO DHF Advisory Committee are those of landing or resting rates for adult mosquitoes as well as larval indices such as the House Index and/or Breteau Index. The
traditional larval indices have several shortcomings. Although it has been shown that there is a strong correlation between the different types of larval index and it is possible to have conversion factors between House, Breteau and container indices, the significance of these indices in terms of abundance of adult mosquitoes and actual man-mosquito contact, and thus their use as tools for forecasting outbreaks or mapping areas of risk, may vary from one area to another. The larval indices give a reasonable distribution and degree of relative prevalence of *A. aegypti*.

The other factors which govern epidemics of DHF are those of vector competence, virulence of the virus and the immunity status of the population as a whole. If vector surveillance is to be used to delineate areas of transmission risk, forecast epidemics and plan intensive control measures, any such surveillance should be carried out in conjunction with assessment of these other factors.

### 7.2 Epidemiological Interpretation of Surveillance Data

It is not possible to give a precise density figure for *A. aegypti* or *A. albopictus* at which no transmission will occur. However, the usefulness and significance of an estimated threshold density cannot be over-emphasized.

Entomologists and health officers should try to work out, in their own localities, possible threshold vector densities required for transmission of dengue haemorrhagic fever. Constant monitoring of this index through routine surveillance enables epidemiologists to predict the trend of the disease and vector control officers to intensify control efforts when the suspected critical level is reached. In actual practice there are, unfortunately, many situations where low vector indices are not maintained and where the disease remains endemic on a year round basis.

As a general guide, the indices are interpreted as follows:

- **Breteau index** less than 5: low risk of transmission
- **Breteau index** more than 50: high risk of transmission
- **House index** more than 10 per cent: high risk of transmission
- **House index** less than 1 per cent: low risk of transmission
- **Biting rate** more than 2 per man hour: high risk of transmission
- **Biting rate** less than 0.2 per man hour: low risk of transmission

After spraying during outbreaks, a parous rate of 10 per cent or less indicates that spraying has been effective and has greatly reduced transmission.

Mosquito surveillance data concerning specific outbreaks of dengue haemorrhagic fever in a few countries are given in Table 2.

It can be seen from this table that, at about the same level of House Index, landing rates vary from three to eight fold (compare the data from Samarang, Basco and Chanthaburi). As the Breteau Index increases, there is an increase in the landing rate. A 2.5-fold increase in the Breteau Index results in about a five-fold increase in landing rate. Increases in the Breteau Index of 3.25-fold and 4.5-fold result in 16- and 25-fold increases in landing rates respectively. The density of the human population, the type of housing and other human factors may influence these measurements considerably.

Landing/biting rates suffer from several drawbacks. They are good tools for measuring the effectiveness of applied control measures, but due to the short flight-range of the mosquito (about 100 metres) their use in surveillance systems requires a very large number of catching stations in order to detect focal concentrations of breeding. The human resources available are generally not large enough for this type of operation and as well the problems of logistics and supervision, when large areas in cities or countryside are to be surveyed, present formidable challenges.

Longevity of the vector has been shown to be important. During the rainy season the longevity and/or feeding frequency may be enhanced. Thus, longevity of mosquitoes, as
Table 2. Adult and larval indices in some areas where DHF/dengue outbreaks have occurred
(A. aegypti, unless otherwise stated)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Larval Indices</th>
<th>Adult Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Brineau</td>
<td>Premises/House</td>
</tr>
<tr>
<td>Singapore (4, 27, 22, 5, 23, 29)</td>
<td>1966</td>
<td>-</td>
<td>14.8 to 29.6 (four areas)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. aegypti</td>
<td>A. albopictus</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>-</td>
<td>9.0</td>
</tr>
<tr>
<td>Menado, Indonesia (40)</td>
<td>1974</td>
<td>43 A. aegypti</td>
<td>27 A. aegypti</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 A. albopictus</td>
<td>6 A. albopictus</td>
</tr>
<tr>
<td>Semarang, Indonesia (47)</td>
<td>1973</td>
<td>122</td>
<td>66</td>
</tr>
<tr>
<td>Baco, Bulacan, Philippines (49)</td>
<td>1973</td>
<td>160</td>
<td>68</td>
</tr>
<tr>
<td>Kuala Lumpur, Malaysia (49)</td>
<td>1973</td>
<td>144</td>
<td>-</td>
</tr>
<tr>
<td>Koh Samui, Thailand (50)</td>
<td>1976</td>
<td>-</td>
<td>63.3 to 100</td>
</tr>
<tr>
<td>Koh Samui, Thailand (51)</td>
<td>1973</td>
<td>180</td>
<td>74.4</td>
</tr>
<tr>
<td>Samut, Central Java, Indonesia (10)</td>
<td>1975</td>
<td>56</td>
<td>50.5</td>
</tr>
</tbody>
</table>

reflected by classifying this according to physiological age, is another important parameter and has not been critically examined in relation to vectors of dengue and their surveillance.

The critical density of A. aegypti, below which transmission will not occur, is still not known in all areas. Chan et al (1977) concluded that, in Singapore, epidemics may occur when the combined Aedes premise index exceeds five per cent. However, these results may not be applicable elsewhere because of different housing conditions.

8. SUSCEPTIBILITY/RESISTANCE TO INSECTICIDES

Susceptibility testing of vectors to routinely used insecticides, e.g., temephos, malathion and fenitrothion, should be regularly carried out. Aedes aegypti has developed slight tolerance to malathion in Thailand. Resistance to pyrethroids has been suspected in Thailand and Singapore. In Singapore and Malaysia, A. aegypti has been reported to be resistant to malathion and temephos, but there are no indications that these insecticides are ineffective under operational field conditions. Table 3 summarizes the information available on resistance of Aedes species to insecticides.

Since organochlorine insecticides are not recommended for the control of A. aegypti, resistance to these compounds is of limited operational significance. However, cross resistance to pyrethroids is possible and could be of importance since these latter compounds have been recommended and used for the control of epidemics of DHF. There is also a risk of development of resistance to insect growth regulators belonging to the methoprene group but, as shown in other culicines, not to diflubenzuron.
Table 3. Insecticide resistance in *Aedes* mosquitoes

<table>
<thead>
<tr>
<th>Species</th>
<th>DDT</th>
<th>Organophosphates</th>
<th>Other compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. aegypti</em></td>
<td>In almost every part of the world in which this species is present, except in certain African countries</td>
<td>Widespread in the Caribbean and neighbouring countries, including South American countries (a, b, c, d, e); India (a), Malaysia (a, b, c, d), New Caledonia (c), Singapore (d), Thailand (d), Viet Nam (d)</td>
<td>Grenada (g), Guyana (g, h), Malaysia (h), South Korea (g), Thailand (h), USA (f)</td>
</tr>
<tr>
<td><em>A. albopictus</em></td>
<td>China, Democratic Kampuchea, India, Indonesia, Japan, Singapore, Thailand, Viet Nam</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>A. polynesianus</em></td>
<td>Fiji, Tahiti and other parts of French Polynesia</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>A. pseudoscutellaris</em></td>
<td>Fiji</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: a = carbamates; b = fenitrothion; c = temephos; d = fenithion e = chlorpyrifos; f = unspecified

Compounds other than the organochlorines and organophosphates are not specified but are given as listed groupings as follows:

- g = carbamates; h = pyrethroids

Present evidence suggests that cross-resistance is to be expected between most carbamates, most pyrethroids, most organophosphates and most formamidines.


These records do not mean that resistance has presented serious control problems.

9. TRANSOVARIAN TRANSMISSION OF DENGUE VIRUSES BY MOSQUITOES

Recently it has been demonstrated that transovarian transmission of all four dengue serotypes occurs in *A. albopictus*. In *A. aegypti* only dengue type 1 has been transmitted transovarially. These findings support the view that, while *A. aegypti* is of major importance from the point of view of transmission of dengue to man, it may be relatively unimportant in the overall natural history of dengue viruses[44].

Note: The monumental work of Sir S. Rickard Christophers, published by Cambridge University Press in 1980, “Aedes aegypti (L). The Yellow Fever Mosquito”, is the most comprehensive account of all aspects of this species, its biology, ecology, distribution and relation to disease. This book should be consulted by those seeking more information.

References


Chapter 9
Vector Control and Intervention
by
Yong H. Bang
Robert J. Tonn

1. INTRODUCTION
In the absence of a safe, effective and economic vaccine against dengue (DF) and dengue haemorrhagic fever (DHF), vector control is at present the only way to prevent the spread of this disease. Throughout tropical zones of member countries of the WHO Regions of South-East Asia and the Western Pacific, Aedes aegypti is widely distributed and in most outbreaks of DF/DHF it is the primary vector. Because of its domestic breeding habitat and dependency on man for a blood meal, this species is an important and efficient vector especially in all the DHF endemic countries in Asia. However, dengue outbreaks have also been attributed in different geographical areas of Asia and the Western Pacific to Aedes albopictus, Aedes polynesiensis, Aedes rondonii, Aedes hebrideus, Aedes cooki, Aedes hakanstoni, Aedes aubae, Aedes niveus, Aedes pseudocutellaris and several species of the scutellaris complex. Unless otherwise mentioned, control of A. aegypti, as the primary vector of DHF in countries of tropical Asia, will be emphasized. Unlike the Americas, where an A. aegypti eradication programme has been developed, control of A. aegypti in tropical Asia was first restricted to airports and seaports as the main concern was the introduction of yellow fever, and only after the first recognition of DHF in the Philippines in 1953 were extensive control measures employed.

The control of A. aegypti and related species has been considered at many scientific meetings, and from 1966 to 1977 it was a priority research topic at several field research units of WHO. Many of the control methodologies developed have been successfully utilized in specialized programmes for the prevention and/or control of DHF, but most are too sophisticated and expensive for operational or routine use by communities. In view of the long-term operational and technical problems associated with the exclusive use of pesticides, WHO has in recent years promoted source reduction and other non-chemical methods for the control of A. aegypti through community participation. Because of increases in numbers of DHF cases, even though case fatality rates have declined, national activities for the control of Aedes vectors need continued support. This chapter reviews various control agents and methods being used or considered for control of A. aegypti during epidemics and inter-epidemic periods of DHF in tropical countries of Asia.
2. STRATEGIES FOR CONTROL OF DENGUE HAEMORRHAGIC FEVER VECTORS

*Aedes aegypti* control measures may be used for routine control or during emergencies. For control of adults, space control equipment is used for rapid killing of flying insects. However, if not complemented with larval control activities, the adult population may soon return to pre-control levels. The World Health Organization (WHO) Technical Advisory Committee on DHF identified different control methods and recommended two different approaches for the control of DHF vectors in countries of tropical Asia: the long-term or routine and emergency control. Vector control strategies need to be developed before the actual operations are carried out to ensure that the proper equipment and pesticides are available and that staff are adequately trained. Any strategy requires knowledge of the vector, disease transmission and control operation conditions.

2.1 Vector Control in Epidemics

The aim of vector control during dengue/DHF epidemics is to kill as many vectors as quickly as possible and to reduce their density sufficiently long enough to interrupt viral transmission. The essence of this control procedure is speed. The main thrust of the action is directed against the adult mosquito as only the adults circulate virus. Management of vector control in an epidemic should be under an interdisciplinary committee with broad powers to rapidly mobilize resources of manpower, spraying equipment, insecticide and transport as well as to plan and direct control operations. In areas with a risk of DHF epidemics it may be of value to stockpile insecticides and equipment. The committee should maintain records of pesticide and equipment supplies in the country. To prevent the spread of DHF outbreaks, space treatment with adulticides should begin immediately after the first few cases have been detected or when there are sound reasons to anticipate an outbreak. If resources are available, it is recommended that the space spray treatments be made to correspond with the mosquito population density and virus transmission or are carried out twice at ten-day intervals either by portable and vehicle-mounted equipment or by aerial application from aircraft, depending upon the size of area and the seriousness of the epidemic situation.

In countries with a rapid surveillance system and in the initial phases of an epidemic in isolated areas, especially if the cases are still scattered, ground space treatment using either ultra-low-volume (ULV) or thermal fog equipment should be made within a minimum distance of 100 metres radius from houses with cases (focal spray). In the national DHF control programme in Indonesia, about 40 per cent of the total input is directed towards focal sprays for epidemics (100 metres radius), but the impact of this approach is not yet clearly known and has been questioned by many scientists because viraemia may have disappeared before the cases have been detected and the epidemic has spread into other risk areas.

Comparative studies on three different methods of vector control with insecticides began in April 1984. No significant reduction of DHF cases was obtained when focal sprays were applied in or near premises of 923 DHF cases reported from 175 "Kelurahan" (villages) with a total population of 3,837,000 in Jakarta (Indonesia). The cost of each focal spray was about US$80. Similarly, no epidemiological impact was observed when focal sprays together with mass ground ULV spraying with malathion twice a year (in April and November) were used in a wider area (500 premises) surrounding reported cases. This combined approach cost about four and a half times as much as the focal sprays alone. On the other hand, case reduction occurred in 45 highly DHF endemic villages, each with an attack rate of 50/100,000 or above, when focal sprays were used in conjunction with two rounds of ULV spraying as well as three cycles of mass larviciding with temephos at three-monthly intervals. This strategy cost nearly 15 times more than the focal spray alone.

Some of the first trials of aerial ULV treatment took place in Thailand during the 1960s. However, actual control of a DHF epidemic by aerial spraying
was demonstrated for the first time in Indonesia in 1973\textsuperscript{10}. Extensive space spraying in DHF endemic countries may effectively curtail DHF epidemics if the measures are applied early in the outbreak\textsuperscript{11}. However, this seldom occurs because of lack of contingency planning including logistics of equipment, insecticides, technical personnel, etc. By the time the measure is implemented it is usually too late to critically assess its value. It is for this reason, plus the cost of supportive treatment of hospitalized cases of DHF, that some type of routine control especially directed against larvae is proposed.

2.2 Vector Control in Inter-epidemic Periods

Source reduction is potentially the ideal method for controlling \textit{A. aegypti}. It requires public motivation through health education and usually legislation and law enforcement to encourage community participation\textsuperscript{1}. In countries of tropical Asia, \textit{Aedes} spp. breed in water stored for domestic use, and environmental measures such as the use of tight covers for water holding containers may control some breeding. Larval control using larvicides or larvivorous fish in domestic water jars should be considered, when necessary, as complementary measures in the context of integrated control management\textsuperscript{12}. In parts of Brazil, \textit{A. aegypti} was at one time eradicated by non-chemical measures such as source reduction\textsuperscript{4}, but once these measures were neglected the mosquito returned.

For the past 20 years, many countries have instituted a national strategy, based on WHO guidance, for reduction of DHF morbidity according to local needs\textsuperscript{2}. With few exceptions, the strategy has been limited to contingency measures such as focal spraying, with or without mass larviciding (Fig. 1). Because of economic and environmental advantages, many DHF endemic countries are operationally or experimentally using non-chemical and integrated control approaches as a strategy\textsuperscript{13}.

In recent years, many countries of Asia have developed contingency plans for DHF epidemic control\textsuperscript{6}. Epidemiological surveillance has improved and coordination between epidemiologists, laboratory services and vector control specialists has been established.

However, there is still room for improvement in monitoring epidemiological and vector status and in development of plans of action for different levels of response. It should be stressed that pre-epidemic or routine control has advantages over attempts to control epidemic situations\textsuperscript{13}. Source reduction campaigns and pesticide treatment of potable and non-potentable water in \textit{A. aegypti} infested areas, as well as advising the public of the dangers of epidemics and the advantages of preventive counter measures, can drastically reduce the impact of an epidemic.

3. CONTROL OF LARVAL DENGUE HAEMORRHAGIC FEVER VECTORS

Larviciding, source reduction, and in some cases the use of bio-control agents, are excellent measures to suppress \textit{A. aegypti} populations. They are valuable in routine control operations, pre-epidemic conditions and as a measure to enhance adult control activities during epidemics. Of these measures, larviciding continues to be the method of choice.

If the various tools available for larval control are used properly, adult populations can be maintained at a level where virus transmission is drastically reduced. Furthermore, larval control is more applicable to community action than is control of adult mosquitoes. Unfortunately, continuous health education and other tools are required to motivate the people. Some cities have successfully used legal measures to promote community involvement\textsuperscript{13}. Larval control requires less equipment and expertise than adult control measures. During epidemics, larval control can be used to support adulticiding and allows people to become involved\textsuperscript{2}.

The major obstacle in larval control is the high cost of larvicide, especially when applied routinely.
to all receptacles which are potential breeding sites for mosquitoes. Field supervision is important to ensure complete coverage of containers. After intensive health education and training, larviciding has been carried out by village volunteers in Thailand, where they have succeeded in reducing the Breteau index by 63-73 per cent with a house coverage of 50 per cent in urban centres and 86 per cent in rural communities. Frequently, because of operational difficulties in treatment of containers, sound management of control resources is as important as the efficacy of control techniques. This is especially true when two different control methods are applied in an integrated vector control approach. In East Africa, a “block system” was tested where a community was divided into zones, containing eight to ten blocks of about 50 houses each\(^{(14)}\). Two-week treatment cycles were made by having one control man treat one block per working day. Any control failure or success in the zone was monitored by the evaluation team assigned to the study.

3.1 Chemical Methods

During the earlier years of DHF epidemics in Asia, control was accomplished through larviciding domestic water jars by treatment with a DDT suspension at a dose of one mg/l, together with five per cent DDT residual spray at two gm/m\(^2\) in premises in the immediate vicinity of DHF cases\(^{(15)}\). Some spraying and fogging were employed around schools and other public buildings. DDT resistance in \(A. aegypti\) was first reported in Asia from Bangkok in 1966\(^{(16)}\). As DHF continued to spread in Thailand, A WHO research unit was established in Bangkok in 1966 with the objective of developing appropriate control methods against DHF vectors. Many larval control agents and methods were tested in different larval habitats as complementary measures to maintain low \(Aedes\) densities especially in areas highly endemic for DHF. From 1966 to 1970, various formulations of temephos were evaluated against \(A. aegypti\) larvae in domestic water containers to determine the residual effectiveness of these formulations in different types of water container and the effect that different water usage practices have on the residual life of temephos\(^{(17)}\). Among all the pesticides examined, temephos was found to be the most suitable for use in drinking water due to its residual effectiveness and lower mammalian toxicity (with an oral lethal dose (LD-50) of 8600 mg/kg).

Treatment with one per cent temephos sand granules (SG) was highly effective when added to water jars at a target dosage of 1.0 ppm (one mg of temephos 1), and a single mass treatment gave good control of \(A. aegypti\) for 6 to 24 weeks, depending upon environmental and cultural factors which appeared to influence recovery of the mosquito density. Temephos was shown to be liberated rapidly from the sand granules, but its toxic properties remained due to a strong affinity of the larvicide for the walls of the containers\(^{(18)}\). Thus, in contrast to the earlier belief, the residual effectiveness of SG is not only due to its slow-release action but also to the nature of the containers themselves, which play a vital role in retaining the larvicide and gradually releasing it. It is effective because the toxicant is liberated from the inner walls of the containers where newly-hatched young larvae browse, even after the container has been frequently emptied and refilled. Therefore, its residual effectiveness is equally good when water containers are treated with emulsifiable concentrate\(^{(19)}\). However, because of absorption, temephos concentrations in water jars are consistently below the treatment dosage. This mechanism may be less visible in metal and plastic containers, whose absorption is usually low. Mass application of temephos SG is easily made by using a calibrated plastic spoon to give dosage at a rate of one gm per ten litre volume of container, whether full of water or not. The sand granules can be pre-packaged to provide controlled treatment by members of a community. Retreatment (as determined in a large-scale field trial in Bangkok in 1970) is required at two-three months intervals or four times a year, depending upon local conditions\(^{(17)}\). Temephos is also highly effective against \(A. samoanus\) when a five per cent emulsifiable concentrate is directly sprayed on to leaf axils of the Pandanus plant using Fontan R-11 mistblowers\(^{(20)}\).
Today, one per cent temephos SG is the only insecticide being operationally used for the control of *A. aegypti* in drinking water. For example, in areas of Indonesia where DHF occurs sporadically (low endemic areas), mass application of temephos at a dosage of one ppm is carried out by trained village volunteers and/or village boy scouts supervised by health centre personnel before the expected highest viral transmission seasons (8). In 1983-84, over 86,000 volunteers treated 2.5 million premises with 111 metric tonnes of one per cent temephos SG, in 1174 villages in 22 provinces (Table 1), covering about 30-40 per cent of the total population in DHF endemic areas. The smallest administrative unit in these areas is the "kelurahan", which consists of 5-600 premises.

Administrative officials are usually elected as honorary campaign managers, and larviciding is technically guided by the paramedical personnel of health centres. Between 1980 and 1985, the global use of temephos in public health services in tropical countries was estimated to be about 200-600 metric tonnes. About 70 per cent of the total input for national DHF control programmes was spent on temephos larviciding (21).

**Table 1.** Number of houses covered with space treatment, larviciding and source reduction in Indonesia, 1974-1985 (in thousands) (8)

<table>
<thead>
<tr>
<th>Financial year</th>
<th>Fogging*</th>
<th>Larviciding*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of houses</td>
<td>Malathion</td>
</tr>
<tr>
<td>1974-75</td>
<td>114</td>
<td>5.8</td>
</tr>
<tr>
<td>1975-76</td>
<td>200</td>
<td>5.5</td>
</tr>
<tr>
<td>1976-77</td>
<td>255</td>
<td>3.1</td>
</tr>
<tr>
<td>1977-78</td>
<td>355</td>
<td>12.0</td>
</tr>
<tr>
<td>1978-79</td>
<td>272</td>
<td>11.4</td>
</tr>
<tr>
<td>1979-80</td>
<td>266</td>
<td>9.6</td>
</tr>
<tr>
<td>1980-81</td>
<td>318</td>
<td>10.0</td>
</tr>
<tr>
<td>1981-82</td>
<td>286</td>
<td>14.5</td>
</tr>
<tr>
<td>1982-83</td>
<td>1,008</td>
<td>14.4</td>
</tr>
<tr>
<td>1983-84</td>
<td>701</td>
<td>24.5</td>
</tr>
<tr>
<td>1984-85</td>
<td>733</td>
<td>15.5</td>
</tr>
</tbody>
</table>

*Amount of 96 per cent malathion used in 1000 litres.
*Amount of one per cent temephos sand granules used in 1000 kg.

No ill effects in spraying personnel or persons drinking water treated with temephos have been reported. Human subjects tolerate levels of 256 mg/man/day for five days or 64 mg/man/day for 28 days without clinical symptoms attributable to temephos. No inhibition of plasma or erythrocytic cholinesterase has been found. No clinical symptoms attributable to temephos were observed among members of a village where domestic water containers were treated monthly with one per cent temephos SG over a 19-month period (22). In most parts of the world, few technical problems with anti-vector measures using temephos have been observed and both adult and larval mosquitoes are still generally susceptible to this organophosphorous (OP) pesticide. However, susceptibility monitoring should be continued.

It has been suggested that more economic larviciding with temephos can be accomplished by carrying out one mass treatment followed by retreatment of only those containers that become positive (Positive Source Treatment) (23). In one such test, the amount of temephos needed was about one-third that of the cyclic mass treatment of all water containers at intervals of three months. However, positive source treatment required about a three-fold increase in man-hours. In Indonesia, paid village workers initially treated water jars with temephos, and this initial larviciding was followed by source reduction through health education involving house-to-house visits by paid workers. In Thailand, larviciding of domestic water containers with temephos in nearly 5000 premises (24) was carried out by 300 school children and boy scouts in Chanthaburi. The house index was reduced from 74.4 per cent to 8.9 per cent and the Breteau index from 180 to 15.5.

Insect growth regulators (IGR) or insect development inhibitors (IDI) have been extensively tested as larval control agents in domestic water containers (12). There is a wide variety of synthetic insect growth regulators in different formulations, including insect juvenile hormones and their analogues and mimics. Some of them (e.g. methoprene) have been on the market for a number of years in the USA but are not as cost-effective as temephos in operational use for control of *A. aegypti* in Asia due to lower residual life and higher cost.
### 3.2 Biological Methods

Biocontrol agents are potentially useful for control of *A. aegypti*, especially *Bacillus thuringiensis* H14 (a spore-forming bacteria) and larvivorous fish. *B. thuringiensis* H-14 was used routinely in about 20 per cent of the total Onchocerciasis Control Programme in Africa, where *Simulium damnosum* resistance to temephos and other OP compounds had occurred\(^{(25)}\). However, the operational use of biological methods for the control of *A. aegypti* in Asia has not yet been considered because of the high operational costs due to the frequent treatments (weekly) which are necessary. Another microbial larvicide, *B. sphaericus* (a complex of aerobic spore-forming strains of bacteria) has some recycling potential. Some of the species in the complex have been isolated from India, Indonesia and Sri Lanka. Unfortunately, compared to *B. thuringiensis* H-14, this microbial agent is less active against *A. aegypti*.

The potential of fish for controlling mosquito vectors seems greater than that of other biological control agents so far studied. Fish were used at the turn of the century in *A. aegypti* control operations in the Americas. Individuals still use them in cisterns and other large water holding containers in the Caribbean. In an isolated village of 3000 inhabitants in China, two omnivorous fish species, *Claris fuscus* and *Tilapia nilotica*, were successfully used in domestic water jars, and in less than two months of the campaign the Breteau index had declined from 123 to 20, and subsequently declined even further\(^{(26)}\). In Male, the capital island of the Maldives, the main *Aedes* breeding sources are wells, rain water tanks and cisterns, in that order. Before chlorination took place in 1978, due to a cholera outbreak, almost all the wells (over 4300) were stocked with larvivorous sea fish of the species *Kulzia taenium*, known locally as "cattafulli". Larval indices for the wells increased from 1.4 per cent to 38.1 per cent soon after chlorination killed all the fish\(^{(27)}\).

In the Pacific Islands, *Coelomomyces* fungus was effective in reducing *A. polynesiensis* breeding in crab holes\(^{(28)}\). However, in general, introductions of fungi are not predictable, and knowledge about release and other technical aspects has not advanced sufficiently to make them a practical control tool.

Larvae of *Toxorhynchites* spp., which feed on mosquito larvae, have been used for experimental control by introduction of either eggs or larvae into containers or of adults into the environment. Recent trials in the USA and St Martin have had promising results, but logistics and mass rearing of the predator make routine control difficult. *Toxorhynchites amboinensis* invaded about 30 per cent of the containers in West Samoa, reducing the larval population by 50 per cent\(^{(25)}\). In Fiji, the population of this same predator controlled 90 per cent of vector breeding in tyres and 60 per cent in tin containers. In French Polynesia, a reduction of 33 per cent in *A. polynesiensis* and *A. aegypti* occurred when *T. amboinensis* was established.

Nematodes of the mermithid group (*Romanomermis culicivora*) have been studied for biocontrol, but they are rather more specific and do not recycle well. Nematodes can be mass reared and may eventually be used in some integrated control activities. Infection of mosquitoes seems to be complex and more information on host-parasite relationships is needed.

A strategy has still not been developed to use these biocontrol agents economically and effectively in an integrated manner with other methods. Much more research is required on the life histories of the mosquito and the biocontrol agent in order to predict the outcome of biocontrol procedures and to plan for the application of such measures.

### 3.3 Autocidal Methods

The autocidal ovitrap designed by Chan *et al.* in Singapore, developed from ovitraps used originally for surveillance of *A. aegypti*\(^{(29)}\), was successfully used as a control device in the eradication of *A. aegypti* from Singapore Paya Lebar International Airport\(^{(30)}\). The effectiveness of autocidal ovitraps was also demonstrated in the control of *A. aegypti* in a semi-rural enclave within the city of Houston (Texas) USA, where the Breteau index at the end of one year of operation had declined by 36 per
cent, in comparison to an increase of nearly 500 per cent in the control area where the traps were not used (31). In Thailand, this autocidal trap was further modified as an auto-larval trap using plastic material available locally, for routine use by Bangkok health authorities (32). Unfortunately, under the local conditions of water storage practices in Thailand, the technique was not very efficient in reducing natural populations of *A. aegypti*. It appears that the successful application of autocidal ovitraps/larval traps depends on their ratio to existing water receptacles in the area under control and hence their attractiveness to females of *A. aegypti*. Better results can be expected if the number of existing potential larval habitats is reduced or more autocidal traps are placed in the area under control, or both activities are carried out simultaneously. It is believed that under certain conditions this technique could be an economical and rapid means of reducing the natural density of adult females as well as serve as a device for monitoring infestations in areas where some reduction in population densities of the vector have already taken place.

3.4 Environmental Methods

The environmental approach to source reduction is labour intensive, but if well planned can reduce *A. aegypti* populations. It may be promoted as a community clean-up campaign or as an anti-*A. aegypti* programme (4). It is important that the activities are well organized and promoted. Since most artificial breeding sites are soon replaced, there is a need for constant source reduction. One method of accomplishing greater involvement on a continual basis is through legal means (33).

Source reduction in permanent drinking water containers can be achieved through using proper fitting lids and screening all openings into the containers through which female mosquitoes might enter. Mosquito breeding surveys should be carried out first to identify the principal sites, and environmental management activities should be designed to control breeding in these sites (34). Proper sanitation and the success of the "Water Decade" could do much to reduce *A. aegypti* populations.

4. SPACE TREATMENT FOR CONTROL OF ADULT DENGUE HAEMORRHAGIC FEVER VECTORS

Space sprays are transient, short-lived treatments with pesticidal fogs, aerosols, and mists, delivered from portable or vehicle-mounted generators or from aircraft (35). This method of treatment is not designed to give a residual pesticide effect, but rather to produce high densities of small pesticide droplets, which, while drifting downwind, will saturate the airspace, penetrating vegetation and buildings to reach both flying and resting mosquitoes. Success depends on producing droplets which are too small to settle readily, and which, while airborne, are available for impaction on all flying mosquitoes or are trapped by filter settlement on their hairs. Success also depends upon repeated treatments to control reinfection by hatching from pupa to adult or by immigration into the treated area (36).

Space treatment operations require a higher level of training and coordination than do residual treatment operations. Monitoring of the vector population should occur throughout the activity to note changes in vector development, distribution and density. This requires training and discipline of field staff as well as the ability to utilize the information obtained from monitoring to change control plans as needed. Since pesticides, especially those applied through space sprays, have some degree of toxicity to non-target organisms, environmental monitoring and safety strategies should be applied (37). Persons operating and maintaining the equipment should have training in safe handling of it as well as in procedures to ensure that the equipment is effective and efficient. The latter requires an understanding of dosage and calibration, droplet size, application timing, application speed and swath, and personnel and public safety.
It is of paramount importance that treatment should coincide with vector density, the daily period of vector activity, and the daily local meteorological conditions as well as with human activity which might disperse the spray or otherwise impede its application\(^7\). For space spraying against *A. aegypti*, the optimum times for outdoor applications are at sunrise (between one hour before and three hours after) and at sunset (from two hours before). Unfortunately, these times correspond to urban traffic rush hours, which can make spraying operations difficult. Ground winds should not exceed 13 km/h or be less than 3 km/h. Treatments should be geared to the incubation of the virus in the mosquito, and treatment intervals planned such that treatment occurs before each brood of mosquitoes becomes infective.

The volume median diameter (VMD) is widely used to measure droplet size for space spraying\(^35\). There are a number of methods in use which determine droplet size, but determination of droplet size is not essential in control programmes as operational experience and programme monitoring determines the success of the work and indicates failures. The distinction between aerosols and mists is based on the distribution of droplet size as follows\(^36\):

<table>
<thead>
<tr>
<th>Sprays</th>
<th>Droplet size in microns (0.0001 cm) (Volume median diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol</td>
<td>Less than 50</td>
</tr>
<tr>
<td>Mists</td>
<td>50-100</td>
</tr>
<tr>
<td>Fine sprays</td>
<td>100-400</td>
</tr>
<tr>
<td>Coarse sprays</td>
<td>More than 400</td>
</tr>
</tbody>
</table>

In earlier control programmes, indoor residual spraying with DDT, dieldrin and other pesticides was the basis of dengue epidemic control in Asia\(^3\). This approach was used in the *A. aegypti* eradication programmes in the Americas. In Malaysia, the container index remained below 2.5 per cent for over 20 weeks after the initial DDT application\(^37\). As resistance occurred, control programmes switched to OP pesticides such as malathion. With the advent of space spraying equipment, residual applications of the OP pesticides were changed to space spray applications. More recently carbamates, pyrethroids and natural pyrethrins have been formulated for space spraying, especially using the ULV application method.

Space spraying equipment should generate a spray near the optimum median diameter range\(^36\). Droplet size varies with nozzle air pressure and condition, pesticide viscosity and flow rate, and operational factors such as type of equipment. Space spraying equipment may require special formulations. For instance, thermal foggers usually require the pesticide to be diluted in an oil, whereas cold mist and aerosol sprays require liquid technical grade or ULV formulations for ULV application and a diluted formulation for low and high volume application.

Space spraying may be used to apply dusts, thermal fogs and ULV cold fogs by portable or vehicle mounted ground equipment or by air. For *A. aegypti* control, either thermal foggers or ULV equipment is used. WHO specifications should be consulted when ordering equipment\(^35\).

### 4.1 Thermal Fogs

The concept of thermal fogging of pesticides originated from the smoke machines which were used during World War II by the US Navy for making ships invisible to the enemy. Thermal fogs are produced by aerosol generators, using heat to volatilize oil solutions of pesticides\(^35\). When injected into the cooler temperature of the atmosphere, the hot oil carrying the insecticide condenses in the form of a fog. Droplet size is generally proportional to the flow rate. The VMD for thermal fog should range from 23 µm to 30 µm.

Thermal fog generators are available in several sizes and are made by a variety of manufacturers. Rates of application range from about 20 to 450 litres per hour. The most common equipment in use in DHF endemic countries of South-East Asia for emergency control of *A. aegypti* is the hand carried thermal fogger, such as the Swing Fog\(^R\). Indonesia has a total of nearly 1000 fog machines and 17 vehicle-mounted cold aerosols (LECO). Some factors to be considered in the selection of fog generators for space treatment are: (i) terrain, (ii) local climatic conditions, (iii) initial and operat-
ing costs, (iv) availability of repair services, (v) size of area to be protected, and (vi) location of the area to be treated, as fog reduces visibility in traffic. Malathion is the pesticide of choice in countries of Asia but other pesticides are also suitable. During the years 1975 to 1985 in Indonesia, over four million premises were sprayed with malathion thermal fogs in DHF control programmes (Table 1). It is important to know if the mosquito is susceptible to the pesticide being used.

In general, thermal fogging is a popular space spray method in areas with high annual rainfall and dense vegetative growth, where the small droplets of fog drift far and penetrate the dense vegetation. A study in Thailand on the control of *A. aegypti* in highly populated urban areas indicated that the cost of thermal fogging was less than that of cold aerosols, and there was no significant difference in suppression of the vector population for a period of two months (Table 2). However, since the droplets are small they are more affected by wind and in some instances may be less effective than ULV applications. Some thermal foggers may be a fire hazard when not properly used. For this reason their use inside homes is cautioned.

Adult mosquito populations usually recover rapidly following treatment by fogging. However, one study showed that combined treatment with thermal fogging and larviciding resulted not only in depression of the adult population for four days but also in prolonged control of larvae in domestic water jars for as long as 24 weeks, thus limiting the recovery of the adult population. Today in Indonesia, mass fogging combined with mass larviciding is common when DHF epidemics occur. In highly endemic areas, focal fogging is usually carried out concurrently with mass larviciding as a preventive measure (Figure 1). In 1980, a DHF outbreak in the city of Palembang in South Sumatra was curtailed by protecting the entire population of 670,000 through the distribution of eight MT of temephos SG using 2500 volunteers to treat water holding containers. This action was followed by two aerial ULV applications of 96 per cent malathion (Figure 2). In Moulmein city in Myanmar, a population of over 200,000 (living in 26,000 premises) was protected through mass malathion fogging (with an SN 100 Fog Generator) and mass larviciding (with temephos SG).

### Table 2. Comparison of different insecticides and equipment for space spraying against *Aedes aegypti* in urban areas of Bangkok, Thailand

<table>
<thead>
<tr>
<th>Insecticide and concentration</th>
<th>Equipment</th>
<th>No. of applications</th>
<th>Dosage rate (g/ha)</th>
<th>Interval (days)</th>
<th>Cost a</th>
<th>Period of effective control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fogging (in diesel oil)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion, 5 per cent</td>
<td>Swingfog</td>
<td>5</td>
<td>0.50</td>
<td>7-15</td>
<td>20.5</td>
<td>2 months</td>
</tr>
<tr>
<td>Dichlorvos, 1.5 per cent</td>
<td>Swingfog</td>
<td>1</td>
<td>0.04 c</td>
<td></td>
<td>10.0</td>
<td>7 days</td>
</tr>
<tr>
<td>Resin 10/10 5</td>
<td>Swingfog</td>
<td>2</td>
<td>0.18±0.23</td>
<td>2</td>
<td>12.5</td>
<td>3 days</td>
</tr>
<tr>
<td>Resin 10/10 6</td>
<td>Swingfog</td>
<td>2</td>
<td>0.18±0.23</td>
<td>2</td>
<td>12.5</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>ULV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion, 96 per cent</td>
<td>Gutbrod</td>
<td>2</td>
<td>0.2-1.3</td>
<td>15</td>
<td>13.8</td>
<td>2.0 months</td>
</tr>
<tr>
<td>Malathion, 96 per cent</td>
<td>Gutbrod</td>
<td>3</td>
<td>0.3-1.2</td>
<td>7</td>
<td>20.4</td>
<td>3.0 months</td>
</tr>
<tr>
<td>Malathion, 96 per cent</td>
<td>Fontan</td>
<td>3</td>
<td>0.3-0.9</td>
<td>13-15</td>
<td>10.0</td>
<td>2.0-2.5 months</td>
</tr>
<tr>
<td>Fenitrothion, 83 per cent</td>
<td>Fontan</td>
<td>1</td>
<td>2.60</td>
<td></td>
<td>18.1</td>
<td>4.0 months</td>
</tr>
<tr>
<td>Fenitrothion, 83 per cent</td>
<td>Fontan</td>
<td>2</td>
<td>0.6-0.8</td>
<td>8</td>
<td>10.5</td>
<td>3.0 months</td>
</tr>
<tr>
<td>Pyrethrin 17 per cent</td>
<td>Mity Moe</td>
<td>2</td>
<td>3-27 c</td>
<td>7</td>
<td>18.1</td>
<td>5 days</td>
</tr>
<tr>
<td>Resin, 10/10 5</td>
<td>Gutbrod</td>
<td>2</td>
<td>3-27 c</td>
<td>7</td>
<td>18.1</td>
<td>No effect</td>
</tr>
</tbody>
</table>

a. Cost of insecticides and solvent used, (US$/ha) as of 1976.
b. 10 per cent bioramethrin w/w and 10 per cent piperonyl butoxide w/w.
c. g/ha.
Figure 1. Prevention and control of dengue haemorrhagic fever in Indonesia – strategy of vector control measures for reduction of morbidity.

- **Epidemic**
  - **Fogging (Mass)**
  - **Mass Larviciding**

- **High Endemic**
  - Higher than 10 cases per 100,000
  - Breteau index: > 20

- **Low Endemic**
  - Less than 10 cases per 100,000
  - Breteau index: < 20

- **Focal Fogging**

- **Source Reduction**
  - **Community Participation**
    - At School
    - Residences
    - Public Places

Figure 2. Control of dengue haemorrhagic fever outbreaks by mass larviciding with temephos sand granules and aerial ultra-low-volume spraying of 96% malathion in Palembang Municipality Area, Indonesia.
4.2 Cold Aerosols

The space spraying of liquid pesticides at a minimum volume of liquid pesticide, usually less than 500 ml/ha, is known as ULV. ULV is generally considered to be the application of aerosols in the 1 to 50 µm VMD range. ULV is directly related to the application volume and not to the droplet size. Nevertheless, droplet size is important and the equipment used should be capable of producing droplets in the 10 to 15 µm range, although the effectiveness changes little when droplet size is extended to 5 to 25 µm. ULV is known as an economic space spray method when compared to high volume (HV) application of water diluted pesticides. The main savings result from: (i) the elimination of diluents; (ii) the elimination of formulation costs; and (iii) the speed of application. As with HV spraying, uniform coverage of the target area by ULV depends on: (i) accurate liquid flow rate; (ii) accurate operation speed; (iii) proper application techniques; and (iv) climatic conditions.

Types of ULV application equipment available at the present time are: (i) portable and vehicle-mounted motorized aerosol generators; (ii) portable and vehicle-mounted motorized mist blowers; (iii) portable and vehicle-mounted thermal foggers; and (iv) aircraft-mounted ULV systems. The type of application equipment needed will depend on: (i) the size of the target area; (ii) the accessibility of target areas; (iii) the vector species to be controlled; (iv) the cost of the equipment; (v) the availability of equipment and maintenance services; and (vi) the safety of operation.

Hand-carried portable equipment

Portable aerosol or mist generators are carried either by hand or by backpack, and are used for small-scale operations as well as in remote areas not accessible to vehicle-mounted equipment. They may also be used inside buildings to kill resting insects. One advantage of portable equipment is that it is more efficient since the aerosol or mist can be sprayed precisely in the target area. However, the equipment has less capacity than vehicle-mounted generators and requires more time per area covered. Furthermore, it is not designed for continuous use and a number of machines may be required for one operation. In a suburb of Bangkok, a portable machine (Mity Moe Sr) was used to apply fenitrothion inside rooms. Two treatments of fenitrothion at a target dosage rate of 0.1 ml/m² of room space, at intervals of about two weeks, produced effective control of A. aegypti for six to seven months.

Knapsack mistblowers

A motorized knapsack mistblower, such as the Fontan R used in many parts of Asia, can produce a swath width of at least 10 m. Earlier mistblowers produced a particle size of between 50-100 µm and were not designed specifically for ULV application, but some could be modified for ULV use. However, newer models may produce much smaller droplets. In Thailand, A. aegypti was controlled with a knapsack mistblower for seven to eight months by sequential application of fenitrothion at a rate of 0.9-1.4 ml/ha at 9-13 day intervals. The same equipment was found useful in the control of A. polynesien sis in villages of Western Samoa, using 96 per cent malathion at a rate of 700 ml/ha in an area surrounded by a chain of high mountains and covered very densely with vegetation. After the first round of spraying no specimens of A. polynesien sis were found for three days. However, the reduction of A. samoanus was not as drastic as that of A. polynesien sis. It has been estimated that one operator can treat up to 100-200 premises per day depending upon their size and the distance separating them. However, due to the weight of the machine and the vibrations caused by the engine, it is recommended that two operators be assigned to one machine.

Vehicle-mounted ULV aerosol generators

Vehicle-mounted equipment is usually preferred to portable generators when an adequate network of roads is available because of convenience in use, reliability, and increased coverage capability. Vehicle-mounted generators are practical for congested areas with good roads. The vehicles are
normally operated at speeds of 8 to 24 km per hour, but applications have been made at speeds of up to 32 km/h with the discharge nozzle directed upwards at an angle of about 45 degrees in such a way that the droplets drift downwards with the breeze. Under good operating conditions, a swath width of 100 to 200m can be expected in urban areas. The flow rate can be adjusted by a flow meter to give the target dosage recommended for the type and concentration of pesticide used and the speed of the vehicle.

Using a LECO-R™ aerosol generator, a 99 per cent reduction of adult A. aegypti density was obtained in Huay Kwang, Bangkok, Thailand. Two treatments of technical grade malathion were used three days apart, at a target dosage of 438 ml/ha, and protected 15,000 inhabitants occupying 1700 premises. One machine treated about 2000 houses per day. The droplet sizes produced by the LECO generators in Huay Kwang were in the range of 1-50 μm.

It is necessary to calibrate the discharge rate, vehicle speed, and swath width to determine the rate of coverage per pass. Training in calibration, operation, servicing and repair of equipment is essential for successful vector control. A good road map of the area showing the treatment route is practical. Sections on the map can be defined to delineate scheduling of cycles. It is ideal to provide one or more knapsack machines per major section to cover areas which the vehicles cannot reach.

Aerial ULV application

Aerial ULV application of pesticide has been tested for control of DHF epidemics. The method was demonstrated for the first time in Semarang, Indonesia in 1973 and later in Menado, Sulawesi in 1974. The method offers several advantages over ground space application: (i) coverage is improved as it is not restricted to road networks; (ii) large target areas can be treated rapidly and efficiently with reduced pesticide application volume; and (iii) fewer operating personnel are needed. However, considerable experience is required for pilots to measure swath widths, speed and altitude over urban areas. A ground crew with two-way radio communication helps.

ULV equipment is not usually commercially available for installation because of the diversity of aircraft. However, many countries have the capacity to carry out ULV application, at least over uncongested areas, because of the availability of agricultural pesticide equipment. Slow flying military aircraft are frequently used when disease outbreaks occur locally. An aerial ULV system consists of: (i) hydraulic nozzles or rotary atomizers; (ii) booms; (iii) pumps; (iv) insecticide tanks; and (v) instrumentation needed for calibration (motor switch, pressure regulator, pressure gauges, flow meter, insecticide screens), which can usually be constructed from locally available products. Remote controlled tachometers are needed for monitoring the rpm of rotary atomizers. However, determination of droplet size upon application and determination of amount of pesticide used per unit of time can provide similar information on dosage rates. It is important to have access to the equipment required at short notice when an epidemic occurs. This may require the Ministry of Health to have an up-to-date inventory of spray aircraft available and to maintain liaison with the military or commercial aerial spraying operators.

For detailed information on the use of aircraft and ULV systems in vector control, references or may be consulted. Although aerial ULV application can be rapid and effective in the control of important vectors of diseases in the case of epidemics, it is important that application is carried out after (i) a thorough planning of the treatments; (ii) calibration of nozzles; (iii) setting of flying height and swath width to give the proper dosage; and (iv) marking of swaths on the ground to give guidance to the pilots. It is also important to use the correct type and formulation of pesticide, as some products are hazardous and others may affect paints on wood and metals. In addition, monitoring on the ground will increase the efficiency and effectiveness of these insecticide applications.

It is estimated that the costs of operating a light spray aircraft are as follows: salaries and allowances (35-45 per cent); insurance on capital
items (10-12 per cent); depreciation (9-12 per cent); hangarage, taxes, licenses (8-10 per cent); and fuel oil (5-6 per cent). The cost of operation can be reduced substantially when the operation is extended to large areas, as has been reported from Tanzania where the costs were US$ 6.90/ha for 20 ha versus US$ 2.00/ha for 2000 ha. In Colombia, the cost of aerial spraying using a rented aircraft was estimated to be about twice as expensive as ground space treatment using a LECOA vehicle-mounted aerosol generator. However, in this case costing was determined by estimating the cost per hectare using agricultural spraying methods rather than those used in public health.

5. LONG-TERM CONTROL

A number of different epidemiological situations occur throughout the geographical range of DHF in Asia. This indicates the urgent need for a long-term control strategy such as that demonstrated in Singapore. The objective of any long-term vector control programme is to reduce the A. aegypti population to the lowest possible level, with the ultimate objective of preventing disease transmission. In tropical Asia the most common larval habitats of A. aegypti are man-made containers. Human behaviour is one of the contributing factors to the continuation of these habitats. Theoretically, the control of DHF can be achieved through an awareness that the breeding habitats created by humans can be modified (source reduction) to eliminate larval breeding. Unfortunately, in many instances the solution is not as practical or easy as it appears.

Source reduction is any planned modification of the environment which reduces or physically removes the water in which mosquitoes develop, or which, through physical changes in the environment, renders the water unsuitable for mosquito production. Because A. aegypti is a container-habitat species, its control by source reduction methods can be more effective than that by other methods. Source reduction measures require continuous action over a long period to achieve effective control. As in Singapore, a certain amount of legislation may be required to promote continued community involvement.

Construction of potable water delivery systems is expensive and generally cannot keep pace with urban population growth. Often, source reduction requires a refuse system capable of routinely removing types of larval habitat. Nevertheless, considerable progress can be made through source reduction. Success of control programmes for A. aegypti by source reduction depend upon operational management skills, including premises inspection, an organized solid waste/refuse service, and permanent commitment to community participation, especially if the programme is to be sustained as a long-term programme.

In Malaysia, urban sanitation through community participation (known as "gotong-royong" campaigns) was a basic control method in a long-term control programme. The programme was intensified by Medical Officers of Health with support from local authorities and the public, first in areas prone to DF/DHF epidemics and then in areas in which A. aegypti was particularly prolific. Under this campaign, the Aedes premises index in priority areas was reduced from 50 per cent in 1973 to 30 per cent in 1975-76 and to 20 per cent in 1978. An example of reduction of DHF without any organized anti-vector intervention is to be found in the Philippines, where a nation-wide campaign for beautification and sanitation by the tourism industry has been operating since 1972.

5.1 Source Reduction (Singapore Case)

The successful environmental measures taken in Singapore, where the house index has been reduced to less than five per cent since 1974 and the resting rate of adult mosquitoes reduced to less than 0.2 females/premises since 1979, are briefly discussed below:

Technical support including vector surveillance: to provide field information required for the planning and implementation of source reduction action, i.e. (i) "where" (high prevalence areas); (ii) "how" (relative importance of various breeding...
habitats); and (iii) "when" (seasonal prevalence of vector breeding).

Control measures: the mechanics of source reduction, as tested in a pilot project in the Geylang area in 1966, are house-to-house inspections by uniformed public health officers to eliminate and destroy breeding sources.

Health education and law enforcement: a month-long campaign of "Keep Singapore Clean and Mosquito Free" was launched in October 1969 to secure public acceptance and support for the "Destruction of Disease Bearing Insect Act" of 1968. This law has provided for tighter and more effective control over those persons whose cooperation is hardly expected with health education alone.

The source reduction activities for *Aedes* control in Singapore were labour intensive, as only about four to eight per cent of the total expenditure was for equipment and materials, while about 88-94 per cent was spent on staff wages including allowances, skill development funds, etc. In the 1977 and 1981 operations, the cost for control of mosquito vectors per capita was calculated to be S$ 2.66 and S$ 3.35 respectively. During the 1978 epidemic, the cost of intensive source reduction was calculated to be about S$ 0.33 for each premises inspected as compared to S$ 0.64/premises for fogging.

5.2 Community-based Control

Organization

In view of operational constraints in larviciding with temephos, the WHO Regional Office for South-East Asia (SEARO) organized a scientific working group\(^5\) and a workshop on community participation in source reduction\(^5\), and developed general guidelines for promotion of community-based control\(^5\). In some DHF endemic countries, health education for source reduction through community participation has been used since the early 1980's as a permanent approach to DHF control in endemic areas. In most instances, this approach began with a limited demonstration project in a few selected areas. In Indonesia, for example, the first source reduction project was set up in 1980 in a small endemic locality of 1000 premises in Surabaya, where volunteers visited all the premises periodically to inspect every possible *Aedes* breeding habitat\(^8\). During the past few years, the concepts and benefits of community involvement have become well accepted and there are a number of ongoing local control programmes with different degrees of success. Since community participation is the central consideration in PHC for the success of any *Aedes* control programme, permanent involvement of the community assumes greater significance as the problem and its solution revolves mostly around man and his environment.

Because of the potential for greater efficiency and efficacy, community-based *Aedes* control projects organized and supervised by local core committees under the technical guidance of local governments are suggested\(^3\). In Semarang (Central Java), a decree of the Provincial Government allowed the mayor to establish a vector control committee in 1982\(^8\). The committee consists of the Chief of the Municipal Health Office (MHO) and the Municipal Education Officer (MEO), as well as medical officers from sub-districts and health centres. A series of health education meetings is usually held at the levels of municipality, sub-district, village, Rukun Kampung (RK)\(^7\), and Rukun Tetangga (RT)\(^7\). A source reduction campaign was organized by the chief of sub-districts, supported by village chiefs, chief RK, RT, social organizations, community leaders, health officers, etc. The technical aspects of the campaign are periodically supported by technical staff from the Provincial Health Department who also provide various health education materials. The weekly community activities include: (i) removal of non-essential artificial water receptacles; (ii) tight covering of water jars; and (iii) refilling and re-emptying (washing) of other larval breeding containers. The weekly activities

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7 RK is a sub-division of the village and consists of 200 families/households. The Chief of RK is a volunteer and is elected by the local community.

8 RT is a sub-division of RK, and consists of 30 families. The Chief of RT is also a volunteer elected by these families.
have been monitored by surveys of containers in 600 premises covering a population of about 34000 (Figure 3). The Breteau index was initially reduced from 57.7 to 33.6 and the house index from 35.6 per cent to 23.4 per cent. After weekly campaigns guided by community leaders, the Breteau index further declined to 15 during a period of two months (Figure 3). The number of reported DHF cases has decreased through this source reduction programme.

In Myanmar, the community-based source reduction campaigns for DHF vector control are usually guided from the central level by political/administrative (party council) and social/voluntary organizations.

Health education

The most common constraint encountered in these community-based programmes is that of motivating the public to continue to perform source reduction over a long period. With this in mind, and following the example of Central Java, each programme usually starts with health education in many municipality areas because people must be made aware that they can do something before any action can occur. Public health education, by definition, aims at bringing scientific knowledge to the people to create an awareness for the betterment of their own health and that of the community.

Unfortunately, little real progress has been made in developing health education methodology to a point that produces and perpetuates the degree of action necessary for long-term control. For example, few studies have been made to determine the reasons why people still store water even when an adequate supply of piped water is available, or why they continually refuse to remove unused water receptacles. For this reason, sociologists and social anthropologists need to become involved in public health research.

However, without legal support, health education alone may be insufficient to produce effective source reduction by community participation, as many other problems prevail in communities related to prejudices and social-cultural practices. Nevertheless, a pilot demonstration in the Phanus Nokhom district of Chonburi province, Thailand, showed reductions in the Breteau index of 60 per cent in the municipality area and of 45 per cent in two rural sub-districts by a source reduction campaign using health education. A similar study has begun in Indonesia, where intensified source reduction programmes were set up on a trial basis in 70 000 premises in 13 provinces in low endemic areas by paid temporary workers. Each worker was asked to make door-to-door visits of 20 premises/day, with four visits/year/premises, for health education and inspection of Aedes breeding sites. It is still too early to report the outcome of this programme. However, for long-term gains a continual input from external forces may be necessary.

School health education

In 50 schools surveyed in five municipalities of Java (Indonesia), over 50 per cent were positive for Aedes breeding, with up to 70 per cent positive in some municipalities. The importance of this with regard to the transmission of DHF resulted in
Law enforcement

Legislation and health education are interdependent and each helps to promote the other, as observed in Bombay, India, and Singapore(7). Almost every large political entity has some legal provisions related to disease vector control, but actual enactment has not been enforced adequately because of lack of local political support. Successful vector control programmes in Bombay(33), protecting a population of over 8.2 million in an area of about 400 Km², have been legally supported by the Bombay Act No. III of 1888, with modification in 1982. Under this Act, legislative measures have been applied for (i) remedial actions in respect of existing mosquito breeding sources such as cisterns, water storage tanks, etc; (ii) regulation of creation of breeding sources; and (iii) penalizing offenders with fines.

In Malaysia(37), to deter householders from allowing mosquitoes to breed on their premises, the “Destruction of Disease Bearing Insects Act” was enacted in 1975 and has been enforced in all major towns. Under this bill, it is an offence to allow the breeding of harmful insects in and around the house, and the penalty ranges from fines to imprisonment.

In Singapore(33), the “Destruction of Disease Bearing Insects Act” of 1968 has been provided with such legal supports as power to (i) enter and examine premises; (ii) prohibit creation of conditions favourable to disease bearing insects; (iii) prohibit the breeding, keeping, distributing or importing/exporting of any disease bearing insects; (iv) direct the owner of premises to take specified measures with regard to disease bearing insects; and (v) arrest, to order to attend court, and to impose penalties (not exceeding one thousand dollars) or imprisonment (for a term not exceeding three months), or both penalty and imprisonment.

The fines collected in 1981 amounted to S$ 317 671 when the total population of Singapore was 2 443 000. Fines were used for promotion of sanitation brochures and other health education material.
6. VECTOR CONTROL EVALUATION

Regardless of the type of control measures used, it is essential to monitor the progress of control programmes with great accuracy and thoroughness. Entomological evaluation is needed, particularly to determine effectiveness and to provide information for making decisions concerning methodology, insecticides, quality and quantity of work, timing of intervention cycles, and other aspects of control. Evaluation can provide assurance of effectiveness or can help demonstrate reasons for failure. When failures do occur, the use of carefully planned and executed evaluation measures will provide data for selecting and planning alternative measures.

Among the Aedes indices commonly used in Asia, the most informative index is that of the indoor resting rate as determined by aspirator catches of females landing/resting on various hanging surfaces. One team of five collectors and one supervisor can cover 30 rooms in different premises within two hours, usually 15 minutes/room. However, because of the difficulties involved in catching adult mosquitoes, it is usual to concentrate on measuring changes in the number of containers and the number of positive larval containers. Ovitrap surveys are more productively carried out when and where adult densities are generally low.

Detailed information on various sampling methods for surveillance and control programmes is presented by Pant and Self. Whatever method is used, samples should be collected at statistically determined, appropriate fixed intervals from houses in different suburbs and areas of towns, from different types of house and from different socio-economic groups in order to obtain samples without bias. Also, to provide comparable data, a consistent method should be used throughout the entire period of the control programme. The total number of samples to be collected depends on the degree of precision required and on the available resources. In Singapore, the number of premises inspected monthly was, on average, 8.3 per cent of the total premises in the control area (with a range of 5.3-11.7 per cent). At the WHO South-East Asia Regional Office Scientific Working Group held in Thailand in December 1983, the following entomological assessment was suggested for a research project.

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**Form: Larval Survey of Aedes Aegypti**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of premises</td>
<td>No. of occupants</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Selecting every third premises up to the number needed to attain a population of 1000 occupants.

$^b$ Subject to source reduction.
on community-oriented Aedes control in an area of 100-800 ha with a population of 100 000 in 20 000 premises: (a) monthly larval surveys in 300 premises, (b) monthly morning indoor adult catches in 72 premises, and (c) weekly ovitrap surveys in 75 premises, each with one indoor trap and one outdoor trap.

7. VECTOR CONTROL STRATEGY

7.1 Planning of Control Strategy

For the planning of DHF control programmes, based on vector control strategies, it is necessary to collect and evaluate basic epidemiological, entomological and other relevant information to determine which control measures should be combined in an integrated manner for the success of the programme(13). The desired goal must first be clearly defined. The information collected should be analyses by experienced professionals for the formulation of a sound and feasible control strategy which will best meet the local conditions, needs and resources(34). The control strategy may be phased, as outlined by Kalra and Bang(34), for a community-based control programme of DHF vectors, as follows:

Preparatory phase

Various basic data to be collected include those on geography, epidemiology, entomology and social/cultural matters as well as those on feasibility, extent of community participation and inter-sectoral action including degree of awareness of the problem in the community and the community’s desires/expectations regarding the proposed control programme.

Planning phase

Based upon the basic information collected in the target area, a working plan should be prepared once the feasibility has been confirmed. The plan of action should be formulated and reviewed in the light of administrative support from various departments and agencies at an inter-sectoral committee where technical details can be worked out on planning, policy formulation, definition of objectives, targets, budgeting, logistics, technical guidance, evaluation, training, inter-sectoral linkage, etc.

Among the detailed activities, the following should be clearly mentioned in the plan of action:

Objectives: the degree of desired control in relation to the time factor.

Control methods: selection of detailed control methods or ways to approach the community for source reduction together with the action to be supported by the Government.

Supportive activities: supportive actions on the parts of individuals, families, the community and the different departments and agencies involved (Table 4).

Community participation: activities that are feasible, identified and agreed to by communities, and the ways and means of approaching the communities to secure implementation (Table 4).

Organization and resource management: organizational set-up for efficient management of the programme specifying each activity and task of each of the operational teams, as in the Singapore operation(19). Very often, the operational team responsible for control intervention is independent of the evaluation team(1).

Logistics and inputs: in addition to the requirements for the programme, there should be rational preparation of control materials and equipment needed to meet any unexpected or emergency situations. The necessary funds, insecticides and equipment should be obtained well in advance. The WHO Technical Advisory Committee(2) has recommended the following to treat a town covering a 20 km² area during an emergency:

- Malathion Technical – 1000 litres (for two applications),
- Vehicle-mounted aerosol generator – one,
- Mist blowers – five, and
- Swing fogs – ten.
Table 4. Plan of action for the control of dengue fever/dengue haemorrhagic fever vectors in tropical Asia

<table>
<thead>
<tr>
<th>Control method</th>
<th>Agent</th>
<th>Activities</th>
<th>Ways and means of approach</th>
</tr>
</thead>
</table>
| Source reduction | Community | 1. removal/reduction of non-essential water containers receptive to mosquito breeding  
2. protection of water containers from larval breeding | 1. health education  
2. mass media (radio, TV, films)  
3. school children/housewives  
4. volunteers  
5. PHC workers  
6. community leaders |
| Source reduction | Government | 1. disposal of refuse  
2. provision of reliable piped water  
3. legislation  
4. monitoring and assessment | 1. set up a core working committee for inter- and intrasectoral coordination  
Same as for source reduction |
| Larval control | Community | 1. larviciding  
2. release of larvivorous fish | Same as for source reduction |
| Larval control | Government | 1. supply of control materials (larvicides and fish) and equipment, as needed. | Same as for source reduction |

**Evaluation:** includes (a) periodical operational assessment to determine the progress of work and actual input received by the programme in terms of materials and manpower, and (b) periodical entomological assessment to determine the success or failure of the control measures applied through vector population and/or epidemiological analysis. When the bulk of the work is being carried out through community participation, it is desirable to have a “built-in” mechanism of cross checking the work.

**Implementation phase**

After collection of the basic information and the drawing up of the working plan for each task, the system of working should be organized for efficient operation of the programme. As in Indonesia\(^\text{(8)}\) and Thailand\(^\text{(3)}\), the programme should then be formally inaugurated by the leaders of the town/city with the participation of community leaders of the different localities/wards and the heads of social/cultural/sports/student and women’s organizations. These prominent citizens should appeal to the public to accept the programme in the interest of their families, and to extend full cooperation for its success. The function should be given wide publicity by the mass media.

In a community-based programme, it is important to give feedback to the community about success or failure, including the benefits occurring from the programme. Such feedback, based upon facts and figures, helps in retaining the faith and continued support of the communities, as was experienced in Singapore\(^\text{(13)}\).

**7.2 National Strategy for Vector Control**

Coordinating efforts by WHO and its member countries have created a viable approach to vector control in DHF endemic areas\(^\text{(2)}\). Vector control in Singapore began in 1966 and has expanded to a successful programme largely through environmental management and legal measures\(^\text{(13)}\). In Indonesia, the national programme has expanded gradually from local operations, and in the past few years has utilized the network of general health services in the context of primary health care (PHC)\(^\text{(8)}\).

The following are examples of national strategies for the control of DHF:

**Myanmar:** Contingency measures since the late 1970s have included focal spraying within a radius of 100 metres around the house of each DHF patient, together with block ULV treatment in a limited area of Yangon, while mass larviciding with one per cent temephos in a number of townships.
in highly endemic states such as Mon and Rakhine has become routine\(^42\). These activities are under the administration of local representatives of townships, and basic health staff implement the measures in accordance with the People's Health Programme, which also includes other vector-borne diseases such as malaria, Japanese encephalitis and filariasis. Health education for source reduction of *A. aegypti* was recently organized throughout the country under the guidance and leadership of local parties and council organizations, and led to voluntary participation through the routine changing or emptying of water containers.

**Indonesia:** The main objectives of the national DHF control programme\(^8\) are: (i) to reduce case fatality through accurate diagnosis, proper case management and early reporting of cases to health centres; and (ii) to control the vector through community participation as guided by volunteers under the primary health care delivery system. All the basic activities for the control and prevention of DHF have been carried out under the network of general health services in the context of PHC. Today, the general activities undertaken for DHF control and prevention through the general public health infrastructure are: (i) monthly reporting of DHF cases; (ii) mass fogging during epidemics or focal fogging by assistant entomologists at Regency CDC units; (iii) mass larviciding and source reduction through community participation or by temporary workers organized and supervised by health centres (Figure 1); (iv) monitoring of vector populations and assessment by entomologists at Provincial CDC/Arbovirus Units; and (v) monthly reporting of DHF control activities through the general system of health services - Regency to Province to CDC/Arbovirus Sub-Directorate. All the activities are reviewed annually and scheduled for implementation, together with allocation of financial and material support, and the whole is coordinated by the Directorate of Communicable Diseases Control and Environmental Health (CDC/EH) in Jakarta.

The control of DHF through anti-vector measures was first organized in 1975, when 105 technicians were trained for ground application of thermal fogs. By 1986, nearly 700 technicians and field supervisors had been trained in the ground operations of insecticidal application for adult mosquito control. In 1985/86, the spending on national and regional training for vector control was about 7.5 per cent of the total budget of the national DHF control programme.

**Malaysia:** Soon after the 1973 outbreak, a plan of action was drawn up by the Ministry of Health to guide all medical officers in taking appropriate action for the prevention and control of DHF in their districts\(^37\). Initially, the main objective of the country-wide control programme was to prepare for possible epidemics, to forestall or minimize the extent of epidemics, and to detect and notify suspected cases as early as possible. Later, the programme was expanded to implement long-term control activities throughout the country. Measures for prevention and control that have been taken include: (i) health education, (ii) source reduction (basic sanitation), (iii) surveillance and early detection of cases, (iv) chemical control, and (v) legislation. In 1977, Malaysia adopted a new strategy of an intensified health education campaign on a national basis, which was enforced with legislation drawn up by an epidemiological committee of the Ministry of Health in 1975.

**Singapore:** The strategy for long-term control of DHF is basically integrated vector control management, similar to that of Malaysia, with source reduction through public health education and law enforcement and, during epidemics, application of ground space spraying\(^13\). This environmentally oriented control programme was started in 1966 by the Vector Control Unit of the Quarantine and Epidemiology Branch, and is the most successful DHF control programme in Asia, being based on the reduction or elimination of the *Aedes* breeding source. This is a specialized programme which uses specially trained teams to make routine house-to-house visits under the supervision of highly qualified vector control specialists. There is a staff of over 1150 in the field operation, covering a population of about 2.5 million. Apart from technical competence, the success is believed to be due to very strict law
enforcement and to vertical control organizations with adequate political and financial support.

**Thailand:** Since 1973, DHF control programmes using anti-vector measures have been implemented in areas of high transmission, and in DHF dissemination points such as schools and hospitals, by DHF and encephalitis control programmes of the Ministry of Public Health, Thailand. In the provinces with low DHF incidence, the control of *A. aegypti* in urban centres is carried out by sanitarians under the supervision of the provincial municipal health officers. In 1982, over six million people were protected by space sprays with 96 per cent malathion or nearly 14 metric tonnes of temephos SG (nearly 14 metric tonnes) was also carried out in 257 communities of 13 provinces.

**References**

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1. INTRODUCTION

There is much interest in the development of dengue vaccine nowadays because of widespread concern over the possibility of new outbreaks of dengue haemorrhagic fever occurring in countries where Aedes aegypti is ubiquitous and where more than one type of dengue virus is circulating. Schlesinger has summarized the theoretical concepts and previous work in dengue vaccine development. He concluded that dengue viruses represent a unique kind of virus where the four types of virus are closely related and yet each is sufficiently distinguishable from the others such that sequential infection may lead to life threatening shock reactions. This situation causes many problems in the development of vaccines.

In this connection it appears that the ultimate goal for dengue vaccine development should be the prevention and control of dengue haemorrhagic fever (DHF) rather than the common, milder form of dengue fever. Since immunity against all four types of dengue virus can be demonstrated in the majority of adults in dengue endemic areas, it should be possible for immunization with appropriate vaccine to elicit broad lifelong protective immunity in persons at risk. Based on the vast experience gained during the development of other viral vaccines, this dengue vaccine should be a polyvalent or tetravalent live attenuated vaccine. The immunity should be protective for all types of dengue virus and should not allow the risk of a natural infection during antibody "waning". The preparation of live attenuated dengue vaccine depends on several biological markers which are indicators of attenuation. The attenuated candidate vaccine is currently prepared either by cloning a mutant which expresses biological markers of attenuation or by passing the wild virus into an unnatural host in serial passages using primary cell lines which are certified as vaccine substrates, such as primary dog kidney cells. The biological markers being used include plaque size (the small plaque is considered to be a covariant with temperature restriction of growth), growth of the virus in human mononuclear phagocytes, suckling mouse neurovirulence and monkey viraemia. The mechanism of these biological markers is at best empirically understood, and the level of attenuation can be assessed on the basis of a comparison between the markers displayed by the wild, original strain of virus and those of the candidate vaccine strains at different passage levels.

Schlesinger's hypothesis was based on a series of studies on mouse passaged dengue virus from which he concluded that there must be at least
two populations of viral particles, one pathogenic to suckling mice only and the other pathogenic for mice of any age. This may imply more genetically heterogeneous populations of virions when considering other biological behaviours (markers). Cloning of a particular plaque of virion (such as a small plaque) with desirable biological markers covariant with attenuated properties should result in an ideal candidate vaccine. This was the approach used for the development of Dengue-2 (DEN-2) vaccine Pri59-S1 clones. The vaccine is a temperature sensitive small plaque variant with a reduced ability to infect and multiply. Experiments on human volunteers yielded a low level of viraemia and some signs and symptoms. At dosage levels of 104 and 105 pfu/ml, the vaccine produced seroconversion in 60 per cent of the flavivirus non-immune volunteers and the antibody level fell to very low levels in six months(2).

2. SYNTHETIC VACCINE

Synthetic dengue subunit peptide vaccine is a remote possibility because of the lack of information on the peptides and their functions and the concern that this kind of vaccine may not produce lifelong immunity. However, new and effective immune modulating substances could make this a serious alternative. There are possibilities that non-structural components of dengue virion, such as NS-1, could serve as protective antigens in addition to the structural ones such as E antigen. Anti-idiotypic vaccine is also a hypothetical candidate.

3. RECOMBINANT VACCINE

Recombinant dengue vaccines seem to be another promising avenue, yet are of medium- to long-term possibility. The advantage of having recombinant genes for protective epitopes packed into a single carrier is apparent. The candidate carriers may include vaccinia virus, where recombinants for other expanded programme on immunization (EPI) and Hepatitis B Vaccines may also be included, 17 D yellow fever vaccine, and Japanese encephalitis (JE) vaccine. The DEN-2 candidate vaccine (16681 PDK 53) may also serve as the carrier for such a recombinant. Intensive activity in this direction, promoted by the World Health Organization (WHO), is taking place in several laboratories around the world. The aim is to obtain information on gene sequencing of different strains and types of dengue virus and to perform epitope mapping of these viruses by using monoclonal antibodies. Molecular sequencing of the genomes of wild and candidate vaccines, such as DEN-2: 16681 - PDK 53, as well as sequencing of the genomes of such viruses at different passage levels, may yield significant insight into the molecular mechanisms of attenuation. All the evidence portends an exciting and enriched period of dengue vaccine development in the near future.

4. RECENT PROGRESS IN THE DEVELOPMENT OF DENGUE HAEMORRHAGIC FEVER VACCINE - POSTSCRIPT 1993

The project to develop a DHF vaccine, which began in 1980 at the WHO Collaborating Centre for Research on Immunopathology of DHF at Mahidol University in Thailand has, by 1993, led to the successful development of a live, attenuated, safe, tetravalent vaccine against all four dengue viruses, which should prevent DHF in target populations in endemic areas. This project, which was undertaken by scientists of Mahidol University, was supported by the Thai government, by WHO, by the governments of Australia and Italy and by the Rockefeller Foundation.

In the years leading up to 1993, four vaccines (monovalent, bivalent, trivalent and tetravalent) were developed. Results of clinical trials with these vaccines, in adult volunteers, have shown them to be safe and to produce encouraging immunological responses. The vaccines have not caused any disabling or untoward effects in volunteers. Studies concerning the genetic stability of vaccine viruses (types 1, 2, 3 and 4) isolated from tetravalent vaccine recipients, seed viruses and parental viruses
have produced satisfactory results and the growth characteristics of each virus have remained constant on passage through mosquitoes.

In August 1992 it was recommended that phase 1 and phase 2 trials of tetravalent vaccines began in school children. The next stage will be to test the vaccine for efficacy under field conditions in large numbers of children at risk, the main objective being to show that the vaccine effectively prevents dengue and DHF in children of the target age group in communities where the diseases are endemic.

The Thai national authorities are taking the necessary action for licensing, regulating and assuring quality control so that the vaccine is available for control programmes within the next three to five years. In January 1993 an agreement was signed between the President of Mahidol University and Pasteur Merieux of France for development and production of the DHF vaccine.

References
About the Book

The dengue virus is responsible for millions of infections, particularly in the South-East Asia, Western Pacific and Americas regions. In the majority of cases the virus causes the self-limiting disease known as dengue fever, although in some situations it may cause the severe condition known as dengue haemorrhagic fever/dengue shock syndrome.

This monograph contains information on the epidemiology, clinical manifestations and characteristics of the virus and on the management, pathology and pathogenesis of the infection. As well, there are sections on diagnostic aids, vaccine development, vector ecology and biornomics, and vector control.

The publication will be useful to medical personnel and to all those scientists, policy makers and administrators interested in the prevention and control of the disease.