

# Dengue Bulletin

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## From the Editor's desk

**E**pidemic dengue fever/dengue haemorrhagic fever (DF/DHF) continues to be a major public health problem globally. The wide geographic spread of both the vectors and the viruses over the last 30 years have led to the development of hyperendemicity in several urban areas in the tropics. Before the 1970s, only nine countries had epidemic DHF. Today, the number has increased several fold. During 2001-2002, the World Health Organization estimated about 50 million infections with over 500,000 cases of DHF with at least 12,000 deaths, mainly among children. A reversal of these trends is the major challenge facing the health communities globally.

Endemic countries are striving hard to improve surveillance, particularly pro-active surveillance which entails collection of blood samples to detect the virus genome and to help predict DHF outbreaks. Cost-effective and sustainable interventions, rapid diagnostics and improved case management to reduce both morbidity and mortality are being pursued vigorously by these countries.

New "innovations" related to: (i) selective indoor residual spray of cryptic resting sites of *Aedes aegypti*, instead of insecticidal fogging, for control of DHF epidemics; (ii) inexpensive and rapid diagnostic test based on the nucleic acid sequence-based amplification (NASBA) as an alternate to PCR which requires specialized training and specific equipment, such as thermocycler, and (iii) detection of dengue virus genome among travellers to South-East Asian countries and its importance as a good predictive for larger epidemics in "travellers contact" countries are the major highlights of this volume.

It is heartening to note that the Dengue Bulletin, which initially focused on WHO's South-East Asia and Western Pacific Regions, has now reached new heights and has gone global. Volume 26, includes contributions from four WHO Regions, viz. South-East Asia, Western Pacific, American and Eastern Mediterranean.

The popularity of the Bulletin can be gauged by the fact that we have closed contributions for Volume 27 which is due for publication by the end of 2003. We are now inviting contributions for Volume 28. The deadline for the receipt of contributions is 31 January 2004. Contributors are requested to follow the instructions carefully while preparing the manuscript. Contributions accompanied by computer diskettes using MS Word for Windows should be sent to the Editor, Dengue Bulletin, WHO/SEARO, Mahatma Gandhi Road, I. P. Estate, Ring Road, New Delhi-110 002, India, or by e-mail as a file attachment to the Editor at [dengue@whosea.org](mailto:dengue@whosea.org).

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# Sero-epidemiological Study of Dengue/Dengue Haemorrhagic Fever in a Metropolitan Hospital in Bangladesh

by

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## Abstract

A collaborative study initiated by the Chittagong Medical College Hospital (CMCH) and the Integrated Control of Vector-Borne Diseases (ICOVED) Project, during September 1996 to June 1997, was a hospital-based descriptive cross-sectional survey in the paediatric age group which identified DF/DHF serologically. The total number of patients were 155 males (60.7%) and 100 females (39.3%), the ratio being 1.5:1 and the mean age 7.1 years. Thirty-five (13.7%) cases were found to be positive for dengue, of which 71.4% were males and 28.5% females; 14.3% were primary, 37.1% secondary and 48.6% mixed primary/secondary. The dengue virus subtypes alone or in combination were: DEN-2: 2.9%, DEN-3: 47.7%, DEN-4: 28.6%, DEN-2+DEN-3: 2.9%, DEN-2+DEN-4: 11.4% and DEN-3+DEN-4: 8.6%. There was no DEN-1 case. Seasonal occurrence of positive cases was: pre-monsoon 28.5%, monsoon 25.7% and post-monsoon 45.7%. Most cases were from thickly populated old quarters of Chittagong city, but 2 cases also were found from far off rural areas of Chittagong. Test results from both labs were in full agreement and no clinical correlation was possible. Contrary to our common notion, dengue is present in Bangladesh with high male preponderance, higher frequency related to monsoon, predominant secondary and mixed types and all serotypes of virus except DEN-1. The present situation is possibly an alarming harbinger of a catastrophe warranting appropriate measures in all relevant spheres.

**Keywords:** Dengue sero-survey, Bangladesh, ICOVED Project.

## Introduction

Dengue fever (DF) is a lesser known infectious disease among the public and

the medical profession in Bangladesh. The classical form of dengue has been known for more than a century in tropical south-East Asia and the Western Pacific region.

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However, dengue haemorrhagic fever (DHF) was reported for the first time in Thailand in 1958<sup>(1)</sup>, in Myanmar in 1970<sup>(2)</sup>, and in India in 1963<sup>(3)</sup>, with regular epidemics and endemicity. These countries geographically surround Bangladesh. On the other hand, Bangladesh is a country where most infectious diseases are prevailing. Of these, vector-borne diseases such as malaria, filariasis and kala-azar are endemic. Dengue is a similar vector-borne disease transmitted by *Aedes aegypti*. All favourable environmental conditions conducive for maintaining such mosquitoes are present here. In clinical practice many febrile infectious cases with features similar to dengue are found without any evidence of bacterial/etiological agent by available tests. In 1964, there was an outbreak of dengue called 'Dhaka fever', which was the first documented outbreak of dengue infection in Bangladesh<sup>(4)</sup> and only one serotype DEN-3 was incriminated. This followed an outbreak in Kolkata (India) in 1963. A few cases of dengue fever were found in 1977-78 in selected areas of Bangladesh by a serological survey done by the Institute of Epidemiological Diseases Research (IEDCR). Some other sporadic studies undertaken with the help of WHO detected evidence of dengue in Dhaka city in the Seventies and Eighties. But no formal documentation was done and, up to 1986, it was thought that four major cities of Bangladesh were free of dengue haemorrhagic fever<sup>(5)</sup>. But over the last decade the scenario has changed. Dengue and DHF have evolved as serious emerging infectious diseases causing high morbidity and significant mortality in almost all countries in south-east Asia.

Bangladesh has an active relationship with all these countries in all sectors, necessitating cross-border communication and travel by people. Thus, there is reason to believe that dengue has a significant presence in the country, which is escaping clinical attention because of the non-availability of appropriate lab facilities and the clinical features of dengue being non-specific.

In view of the above, the Directorate-General of Health Services, in collaboration with WHO, included a formal sero-epidemiological survey of DF and DHF as one of the components of the Integrated Vector-Borne Diseases Control Project (ICOVED). The aims of this survey were: (i) to identify the proportion of dengue/DHF amongst patients aged 1–15 years attending the Chittagong Medical College Hospital; and (ii) to attempt clinical correlation, if possible.

## Materials and Methods

This hospital-based descriptive cross-sectional survey (September 1996–June 1997) was based on random accidental sampling to define and identify eligible patients suspected of DF/DHF. Paired serum samples from each index case were taken. The *first* sample on attendance at the outpatient department was accepted only after confirmation as per inclusion/exclusion criteria after 48 hours. The *second* sample was taken after seven days of the first sample. The Chittagong Metropolis was chosen for the study and the centre was the Chittagong Medical College Hospital, the only tertiary health care delivery centre covering the

population of the city, the suburbs and the adjoining hill districts.

**Case inclusion criteria:** 1-15 years of age, febrile illness for 72 hours, no focal clinical signs within next 48 hours, chest X-ray (CXRPA), complete blood count (CBC), urine routine examination (URE) negative for any other infectious diseases, and negative blood slide examination for malaria parasite.

**Case exclusion criteria:** Clinical review and routine tests revealing any confirmatory evidence of other infectious diseases, refusal to give consent and or samples plus lost from follow-up.

**Serological diagnosis and tests:** The diagnosis was done with serological test, the haemagglutination inhibition test (HIT) as per 'Clarke & Casals Technique 1958'<sup>(6)</sup>, which was done at the Virology Laboratory of IEDCR. A total of 152 samples were cross-checked at the Armed Forces Research Institute of Medical Science (AFRIMS) in Bangkok, Thailand.

**Sample collection and transportation:** Samples were collected and stored at -20°C and were bulk transported to IEDCR for testing.

**Data collection and analysis:** A specified data collection form was used for each case. The data was analysed with EPI6 software following the standard format.

## Results

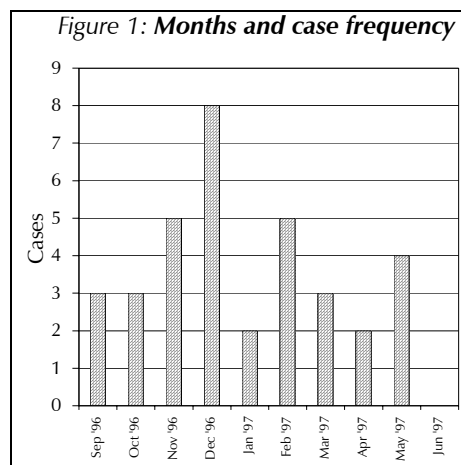
A total of 283 cases were screened, out of which 255 cases with a mean age of 7.1 years were included for the study,

generating the same numbers of paired sera. Out of these, 35 (13.7%) cases were found to be positive by HI test, of which 25 were males and 10 females (Table 1).

**Table 1: Patients and HI test profile**

Parameters	Results
Cases included for the study	Total 255, male 155 (60.7%), female 100 (39.3%). Age: mean 7.1±2.8 years, maximum 15.0, minimum 2.0, mode & median 7.0, 95%CI 6.76-7.44
HI positive cases	Total 35, male 25 (71.4%), female 10 (28.5%). Age: mean 7.1±2.8 years, maximum 15, minimum 2.0, median and mode 7.0, 95%CI 6.17-8.03
Viral sero-types	D <sub>1</sub> : 00(0.0%), D <sub>2</sub> :01(2.9%), D <sub>3</sub> :16(47.7%), D <sub>4</sub> :10(28.6%), D <sub>2</sub> +D <sub>3</sub> : 01(02.9%), D <sub>2</sub> +D <sub>4</sub> :04(11.4%), D <sub>3</sub> +D <sub>4</sub> :03(08.6%)
Infection types	Primary:05(14.3%), secondary: 13(37.1%), primary/secondary: 17(48.6%)

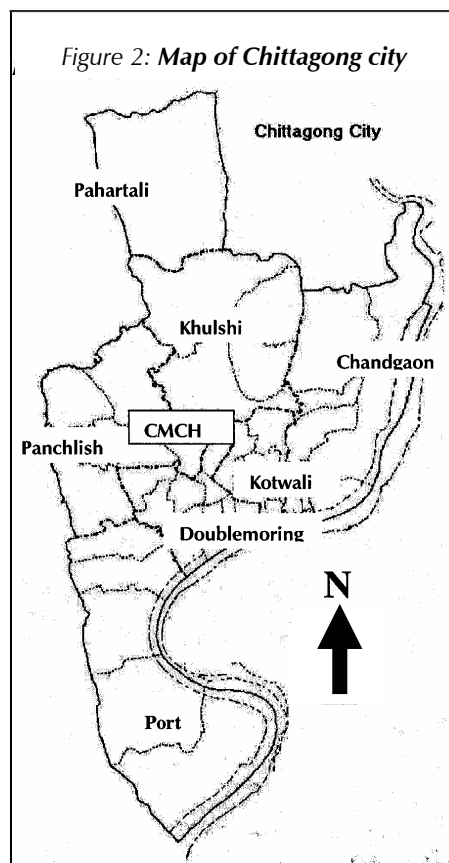
The sample collection did not spread over the whole year and all the seasons as it was done only from September 1996 to June 1997. Roughly, the collection covered a part of the monsoon, post-monsoon and pre-monsoon periods. The positive cases showed higher frequencies during the pre- and post-monsoon periods. But the highest yield was found during the autumn and winter periods (Figure 1).



The area-wise distribution of cases showed that 33 cases came from different zones of Chittagong city and one each from a contiguous and a non-contiguous rural area. But most cases were from the thickly populated old quarters of the city (Table 2 and Figure 2)

**Table 2:** Area-wise frequency distribution of positive cases

Zone	Cases	Comments
Kotwali	17 (48.5%)	Old quarter, thick population
Pahartali	05 (14.2%)	Old quarter, thick population
Doublemoring	04 (11.5%)	New quarter, less thick population
Panchlish	04 (11.5%)	New quarter, less thick population
Chandgaon	03 (08.5%)	New quarter, less thick population
Anowara	01 (02.8%)	Contiguous rural area
Chandanish	01 (02.8%)	Non-contiguous rural area



The clinical and lab features, including patterns of haemorrhagic features and their frequencies, were compared between the positive and negative groups, which was found to be non-significant (Table 3).

Of the lot, 152 paired samples were cross-checked at AFRIMS, Bangkok, Thailand. The results were in complete agreement with those of IEDCR, Dhaka ( $\chi = 1.0$ ).

**Table 3:** Haemorrhagic features comparison

Haemorrhagic features	Positive group (N=35)	Negative group (N=230)
Ecchymosis	00 (00.0%)	00 (00.0%)
Epistaxis	01 (02.9%)	00 (00.0%)
Gum bleeding	00 (00.0%)	00 (00.0%)
Hematokezia*	00 (00.0%)	00 (00.0%)
Hematuria	00 (00.0%)	00 (00.0%)
Hemoptysis	01 (02.9%)	00 (00.0%)
Melena	00 (00.0%)	01 (00.5%)
Rash	03 (08.6%)	20 (09.1%)
Red eyes	02 (05.7%)	13 (05.9%)
Tourniquet test	00 (00.0%)	00 (00.0%)

## Discussion

This study revealed that dengue infection was present in the country among a significant proportion of febrile patients. The presence of more secondary responses pointed to the fact that the transmission was continuing. Moreover, the evidence of more than one viral serotypes should be taken as a warning that outbreaks of DHF might occur in the future<sup>(7)</sup>. The interpretation of the study was based on the WHO criteria for HI test, so there might be some lacunae of cross-reaction by other *falvivirus* infection as evident through some equivocal test results. However, the overall interpretation was that a low-grade infection and transmission was present.

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\* Hematokezia is unaltered per anal bleeding in contrast to melena.

This should warn the health authorities and policy-makers that dengue outbreaks might occur in the future. From the experiences of other dengue-endemic countries an inference can be drawn that a similar situation might occur in Bangladesh as well<sup>(8)</sup>.

The clinical correlation was not possible as clinicians were not acquainted with the presentation and management of dengue cases. Thus in this study, some subtle changes were perhaps ignored which one would take into account after becoming conversant with the condition. During this period a serious dengue outbreak was occurring in neighbouring India (1996)<sup>(9)</sup> and in Thailand (1997). There might have been a linkage with the outbreak in India as there is intense population exchange between that country and Bangladesh, not to speak of only the city of the study. The outbreak in Chittagong possibly was of low grade and therefore without any severe syndrome to draw attention by visible characteristic features. But covert changes may, in future, lead to cases with severe characteristic features.

Most cases were from the old quarters of the city which have a high population density. This reaffirms the role of the predisposing factors for the breeding and sustenance of the vector. These factors are not only present but are constantly increasing. The real concern, however, is the reporting of cases from rural areas; though it is not unusual for dengue to spread to rural areas.

The full matching of test results made by IEDCR and AFRIMS suggests that if appropriate measures are taken, efficient capacity for lab diagnosis can be built within Bangladesh. In fact, confirmatory specific lab test is neither essential nor is needed for guiding the clinical management of dengue. But these tests are important for the epidemiological and control programmes. Ideally, sentinel surveillance should be continued where these tests become necessary.

From this study it can be concluded that dengue infection is present in Bangladesh, and that its outbreaks may occur any time, and DHF may also take the usual toll of life. Therefore, national programmes should address the issue appropriately in all its aspects such as diagnosis and clinical management, continued surveillance, capacity-building

for lab confirmation and viral isolation. Moreover, linkages and collaboration should be developed with countries and centres conversant with this public health problem by appropriate utilization of WHO portals and channels.

## Acknowledgement

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# Dengue Control in North Queensland, Australia: Case Recognition and Selective Indoor Residual Spraying

by

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## Abstract

A large epidemic of dengue in Townsville and Charters Towers, north Queensland, Australia, initiated the development of a Dengue Fever Management Plan for north Queensland (DFMP) in 1994. The DFMP integrated disease surveillance, vector control and health promotion activities to prevent dengue outbreaks. While initially successful in preventing the recurrence of epidemic dengue in north Queensland, a protracted epidemic of DEN-3 in the Cairns area led to a revision of the DFMP in 2000. The revised DFMP placed emphasis on the early recognition of cases and development of a specialized Dengue Action Response Team (DART) to conduct selective indoor residual spraying with pyrethroid insecticides to control the vector, *Aedes aegypti*. Since the launch of the DFMP 2000, dengue outbreaks have been contained in terms of the areas affected, duration of the outbreak and the total number of cases despite an increase in recognized dengue activity.

**Keywords:** Dengue epidemic, DEN-3, Dengue Action Response Team, selective indoor residual spray, *Aedes aegypti*, Australia.

## Introduction

North Queensland, Australia, has been subjected to outbreaks of dengue since the late 19th century<sup>(1)</sup>. Following a large outbreak of DEN-1 in 1981, an *Aedes aegypti* control programme was implemented in north Queensland between 1982 and 1985<sup>(2)</sup>. Although the populations

of *Ae. aegypti* were reduced, funding for the programme ceased in 1985. Coincidentally, international airports were opened in the cities of Cairns and, later, Townsville in north Queensland at about that time. This increased the likelihood of viraemic travellers arriving from endemic countries directly into urban centres in the dengue receptive region of Australia.

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Therefore, it was not surprising that, with no organized dengue control programme in place, an unrecognized importation of DEN-2 into Townsville in 1992 led to an explosive (and very large) epidemic in that city<sup>(3)</sup>. It ultimately spread to the nearby town of Charters Towers, where an estimated 26% of the population was infected<sup>(4)</sup>.

## **The 1994 Dengue Fever Management Plan for north Queensland**

Following the 1992-93 dengue epidemics, Queensland Health, in collaboration with Dr Duane G Gubler of the Centers for Disease Control and Prevention, along with a range of stakeholders including local government, medical, laboratory and academics, developed a structured Dengue Fever Management Plan (DFMP) for north Queensland. The plan detailed integrated strategies, namely, disease surveillance, vector control and health promotion, to prevent dengue outbreaks and to ensure that the virus was eliminated from north Queensland, preferably before local transmission had occurred. Underpinning the plan was the need for laboratory surveillance for the early detection of cases, particularly imported ones, followed up with rapid and thorough vector control. The ultimate success of the plan relied upon successful vector control through a collaborative effort between local government environmental health officers and Queensland Health personnel. The strategies and the collaborative efforts successfully limited an outbreak of DEN-2 in Cairns in 1995 to only four cases<sup>(5)</sup>.

As an alternative to outdoor ultra-low volume spraying, selective indoor residual

spraying of households of cases was first utilized in north Queensland during a large outbreak of DEN-2 in the Torres Strait in 1996-97<sup>(6)</sup>. *Ae. aegypti* harbours in dark, sheltered areas such as under beds and tables and inside closets in premises<sup>(7)</sup>. Selectively treating these sites with a residual pyrethroid insecticide, such as deltamethrin or lambda-cyhalothrin, effectively reduced *Ae. aegypti* activity, and therefore dengue virus transmission, in foci of dengue activity during the outbreak<sup>(8,9)</sup>. Furthermore, the residual activity of lambda-cyhalothrin is high. In a field bioassay, 10 female *Ae. aegypti* in WHO bioassay cones were exposed to wood and a cotton/polyester cloth treated with lambda-cyhalothrin (Demand SC 2.5% AI diluted at 16 ml/litre water) for 10 minutes; over 90% mortality was achieved up to 45 days post-treatment (S Ritchie and S Long, unpublished data).

## **The 1997-1999 DEN-3 Epidemic**

Although the strategies in the 1994 DFMP had been satisfactory for nearly five years, a large and protracted epidemic of DEN-3 in Cairns, Port Douglas and Mossman in 1997-1999 proved very difficult to control<sup>(8)</sup>. The epidemic demonstrated that the dengue virus could spread very rapidly via the movements of viraemic individuals to initiate multiple foci of the disease that could not be adequately controlled using conventional methods. It also demonstrated the importance of 'ignition' premises, such as backpacker hostels, that catered to a rapid turnover of a high volume population of travellers who could import the virus, and of 'dissemination' premises, such as schools, that could lead to the rapid dispersal of the dengue virus throughout a community via

infected students and staff. Cryptic breeding sites, including subterranean sites such as sump pits<sup>(10)</sup> and elevated roof gutters<sup>(11)</sup>, were an important source of *Ae. aegypti*. Not surprisingly perhaps, the DEN-3 virus evaded eradication, and eventually this led to staff 'burnout' and staff shortages that, in turn, severely hampered the vector control activities. During the outbreak, it was demonstrated that a single treatment of *Ae. aegypti* resting places<sup>(7)</sup> (e.g. dark, protected areas such as under tables and beds, inside wardrobes, etc.) inside premises using lambda-cyhalothrin (Demand SC, 2.5% AI at 16 ml/litre water) effectively reduced *Ae. aegypti* populations; the mean number of *Ae. aegypti* eggs per ovitrap in treated vs. untreated areas was 2.2 vs. 12.5, respectively, a significant ( $P < 0.05$ ) difference<sup>(8)</sup>.

## The Dengue Action Response Team

In December 1998, Queensland Health established the Dengue Action Response Team (DART), a trained specialist team of three personnel. The sole responsibility of the DART was to implement *Ae. aegypti* prevention and control strategies, including, where necessary, the selective indoor residual spraying of premises. The DEN-3 epidemic ended within three months of the DART commencing activities.

## Elements of the current DF management plan for north Queensland

In 2000, a revised DFMP ("Dengue Fever Management Plan for North Queensland,

2000-2005") (DFMP 2000) was launched to incorporate new strategies, including those to be implemented by the DART. The objectives of the DFMP 2000 are: (i) to recognize dengue cases as rapidly as possible through not only laboratory but also clinical surveillance; (ii) to respond to dengue cases, both imported and locally-acquired, with thorough and sustained vector control aimed at eliminating the dengue virus before it can spread to other urban foci; and (iii) to use a variety of education initiatives to maintain community awareness of dengue, its mode of transmission, and the need for individuals to take action to prevent *Ae. aegypti* breeding in households and business premises.

## Disease surveillance

Between December 1994 and May 2002, there were 69 notifications of the importation of dengue where the traveller was viraemic (and therefore infectious to *Ae. aegypti*) while in north Queensland. All four serotypes of the dengue virus were imported during this time. The numerous outbreaks in the 1990s (Figure) reflect the necessity to identify imported viraemic cases as quickly as possible. Dengue is a notifiable disease in Australia, and as such, the Tropical Public Health Unit (TPHU) should be notified of a case by laboratories or physicians. Because many imported viraemic cases are not promptly notified to the TPHU<sup>(12)</sup>, local *Ae. aegypti* may be infected and on the wing before control measures can be initiated. Furthermore, several of the dengue outbreaks had no known origin, indicating that many imported dengue cases go unrecognized.

Although serologically false positive results remain a problem, the timeliness of



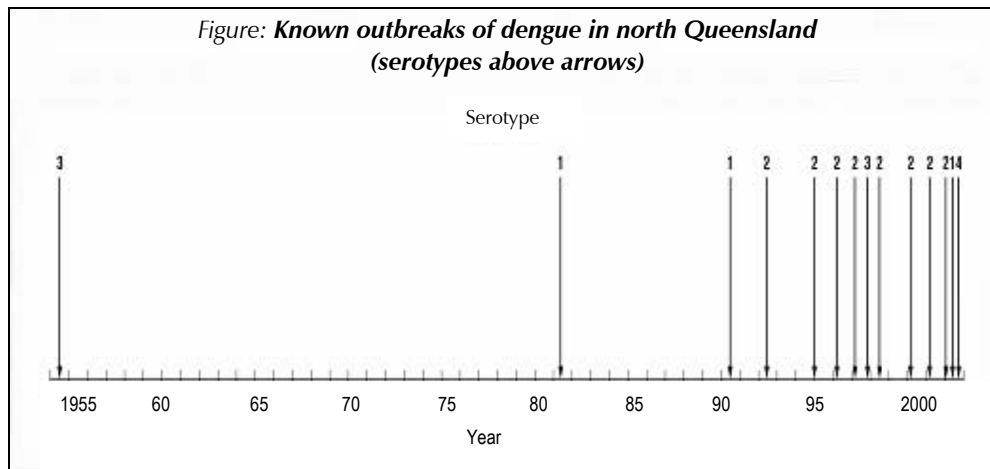
laboratory testing for dengue has improved in recent years. Serological tests have been augmented by rapid immunochromatographic card or dipstick tests, while the polymerase chain reaction test has supplanted virus culture to detect dengue virus in serum, giving quicker results yet still providing valuable genetic sequencing information. Finally, careful patient interviews by public health nurses are needed to establish the patient's travel history, particularly whether a case is imported or locally acquired, and points of contact where the patient may have infected mosquitoes.

## Vector control

Upon the notification of either a dengue IgM +ve test result or a suspected imported case, the DART makes an immediate response. The points of contact (usually the case's residence and place of work) are mapped, and the intended vector control activities detailed. The DART conducts selective indoor residual spraying and larval

control/source reduction activities within 100 metres of a contact point, while local government personnel conduct larval control in a zone 100-200 m from these premises. Larval control includes removal of small containers while larger containers are treated with S-methoprene pellets<sup>(13)</sup> or sprayed with aerosol surface sprays<sup>(14)</sup>. Particular care is placed upon the identification and treatment of cryptic subterranean<sup>(10)</sup> and elevated<sup>(9,11)</sup> breeding sites, such as sump pits and roof gutters, respectively. If multiple dengue cases are reported in a particular area, the response zone is expanded. Field data are recorded onto a palm-top computer, then imported onto a Geographical Information System (GIS) for mapping response activities, the latter particularly useful for follow-up treatments.

Between outbreaks, the DART conducts preventive larval control activities at ignition and dissemination premises, such as backpacker accommodation and schools, respectively.



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## Health promotion

Health promotion plays two critical roles in the DFMP 2000. Firstly, during a dengue outbreak, publicity in the form of media advisories, delivered by local media, are used to inform the public about areas with active dengue transmission. Residents are advised to take precautions by clearing households and backyards of any obvious breeding sites, using surface sprays to kill adult *Ae. aegypti* and taking personal protective measures to avoid being bitten. Secondly, a television-based mass media campaign is run on a paid schedule during the wet season to educate the public about dengue<sup>(15)</sup>. The domestic habits of the vector and how to control it are discussed, along with symptoms and consequences of the disease. Programmes to educate school children have also been developed.

## Success of the DFMP 2000

Since the implementation of the DFMP 2000, five outbreaks of dengue that occurred in north Queensland have all been

relatively easily contained, and very little locally-acquired dengue has been detected beyond the initial focus of activity (Tables 1, 2). Furthermore, the number of cases acquired after the initiation of control efforts has been limited and the duration of outbreaks reduced. The latter is critical in helping to maintain a focused effort by dengue control staff; the burnout during the 70-week DEN-3 epidemic in 1997-99 contributed to the duration and spread of the outbreak<sup>(8)</sup>. We consider that early case recognition, coupled with selective indoor residual spraying of cryptic resting sites and intense larval control efforts, are instrumental in the success of the plan to date.

The utility of the current DFMP was also evident in early 2000 when approximately 2000 Australian Defence Force personnel and aid workers returned from duty in East Timor to north Queensland. Despite at least eight viraemic importations of dengue occurring in these personnel, focused and collaborative responses ensured that not one of these cases led to subsequent local transmission<sup>(9)</sup>.

**Table 1:** Dengue outbreaks in north Queensland before the revised (2000) Dengue Fever Management Plan for north Queensland

Dengue serotype	Affected areas, dates (reference)	Duration (weeks)*	Cases before control measures	Cases after control measures began	Total no. of cases	No. of foci of transmission
2	Cairns, Feb 1995 <sup>(5)</sup>	6	1	3	4	1
2	Torres Strait, Dec 96-Feb 97 <sup>(6)</sup>	29	49	159	208	6
2	Cairns, Dec 97-Feb 98 <sup>(9)</sup>	11	6	6	12	3
3	Cairns, Port Douglas, Mossman Dec 97-March 99 <sup>(8)</sup>	70	20	478	498	15

\* Time in weeks from onset of first case until onset of last known case.

**Table 2:** Dengue outbreaks in north Queensland after the revised (2000) Dengue Fever Management Plan for north Queensland

Dengue serotype	Affected areas, dates (reference)	Duration (weeks)*	Cases before control measures	Cases after control measures	Total no. of cases	No. of foci of transmission
2	Cairns, Feb 2000 <sup>(9)</sup>	6.7	17	33	50	1
2	Townsville, April-May 2001 <sup>(16)</sup>	3.2	09	0	09	2
2	Kuranda, March-April 2002	9.0	18	3	21	1
1	Townsville, April 2002	0.4	02	0	02	1
4	Cairns, May 2002	3.0	02	0	02	1

\* Time in weeks from onset of first case until onset known case.

## Future challenges

A substantial risk of outbreaks of dengue in north Queensland remains despite the success of the DFMP 2000. Up to June, three albeit small outbreaks occurred in 2002 in north Queensland (Figure). Significantly, they were all of different origin and, indeed, involved different serotypes of dengue viruses (DEN 1, 2 and 4). In addition, over the same five months, there were five viraemic importations notified. None of these importations was associated with the three outbreaks; it is likely that significant numbers of importations go unrecognized. Can the intensity demanded by the DFMP, and the resurgence of dengue activity since 2000, be maintained by DART and other TPHU staff? Will the public get fatigued of the dengue education campaigns and outbreak alerts? Will selective indoor residual spraying lead to insecticide resistance in *Ae. aegypti* and can novel

control methods, such as removal trapping, be used to counter insecticide resistance? These are some of the issues that will challenge health staff in their efforts to continue to control dengue in north Queensland.

## Acknowledgement

We are indebted to those who contributed to the formulation of the DFMP for north Queensland, particularly Drs Duane Gubler and Paul Reiter of the Centers for Disease Control and Prevention, and to Dr Goh Kee Tai of the Ministry of the Environment, Singapore. We acknowledge the efforts of TPHU medical, entomological and environmental health staff who have strived to make the DFMP work, and thank Ann Richards for providing data and Di James for the preparation of the figure.

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# Status of Dengue Control Programme in Taiwan – 2001

by

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## Abstract

Taiwan is a dengue-epidemic area and epidemiological studies have shown that most indigenous dengue cases can be traced to imported cases preceding the local transmission. Thus, the critical issue in dengue prevention and control is to identify the index case of imported dengue patient, then break the dengue virus transmission as quickly as possible. The Dengue Prevention and Control Centre, which is responsible for dengue control in Taiwan, is a joint programme organized by the Department of Health and the Environmental Protection Administration, with the participation of both the Central and local governments, started at 1988. There are seven working groups in the centre which deal with various affairs including epidemiology, entomology, insecticide application, virology, medical care, source reduction and health education for total coordination. To further strengthen this programme, international collaboration in surveillance, epidemic information exchange, as well as understanding the pathogenesis of dengue haemorrhagic fever and dengue shock syndrome and prevention of the disease process is necessary.

**Keywords:** Dengue virus, mosquito surveillance, mosquito control, law enforcement, indigenous dengue case, health education, Taiwan.

## Dengue in Taiwan

*Aedes aegypti* and *Aedes albopictus* are the two main vectors involved in the transmission of dengue in Taiwan. The former is active in indoor settings, primarily distributed between the south of Putai and the north of Hengchun below 1 000 m, and

the latter is found outdoors and is widely distributed in the plains and mountainous regions up to an altitude of 1,500 m throughout Taiwan. As a day-biting mosquito that prefers to feed on humans, *Aedes aegypti* is responsible for most epidemics. Several outbreaks of dengue fever were

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recorded in Taiwan even before World War II. No dengue case was reported since 1945 until 1981 when a DEN-2 outbreak occurred in Liouchiou Township of Pingtung County. In 1987, a DEN-1 outbreak occurred in the southern part of Taiwan. The major outbreaks of dengue fever in Taiwan are included in Table 1. Dengue haemorrhagic fever cases were taken into account since 1994. An analysis of the monthly frequency of indigenous dengue cases shows that it peaks around September-December, which is quite different from that in south-east Asia<sup>(1)</sup>. Local strains of all four dengue serotypes were isolated from various regions of Taiwan as shown in Figure 1. Using the year 2000 as an example, various serotypes of dengue virus were isolated from imported and/or indigenous cases (Table 2). While one DEN-3 virus was isolated from imported cases, one DEN-3 and 10 DEN-4 viruses were isolated from indigenous cases. Although the isolation rate is low, probably due to the timing of sample collection, it is difficult to isolate dengue virus from blood samples collected beyond seven days post-fever onset. The dengue virus isolation provides important information for our dengue control programme. The characteristic of dengue virus infection in Taiwan is that most indigenous dengue cases can be traced back to imported cases preceding the local infection, although the issue of "Whether dengue is endemic in Taiwan" is debated. The control strategy is based on the assumption that dengue transmission is from an imported case as its success indicates that dengue virus is primarily imported into Taiwan island every year.

**Table 1:** Major outbreaks of dengue fever in Taiwan, 1981-2001

Year	Index case	Epidemic area	Infection rate	Serotype
1981	Liouchiou	Liouchiou	>80%	DEN-2
1987	Pingtung, Kaohsiung	South	13%	DEN-1, 2, 4
1988	Pingtung, Kaohsiung	South	–	DEN-1, 3, 4
1989	Pingtung, Kaohsiung	South	–	DEN-1, 2, 4
1991	Kaohsiung	Kaohsiung	–	DEN-1, 3
1994	Kaohsiung county	South	–	DEN-1, 3
1995	Taipei county,	North, Middle, South	–	DEN-1,2,3
1996	Taipei	Taipei, Kaohsiung	–	DEN-1, 3
1997	Tainan city	Tainan city	–	DEN-2
1998	Kaohsiung city Tainan city	Kaohsiung city Tainan city	–	DEN-2 DEN-3
1999	Kaohsiung	Kaohsiung	–	DEN-1
2000	Tainan city	Tainan city	–	DEN-4
2001	Kaohsiung city	Kaohsiung city	–	DEN-2

– = Information not available

**Table 2.** Virus isolation by countries and from imported and indigenous dengue case during 2000

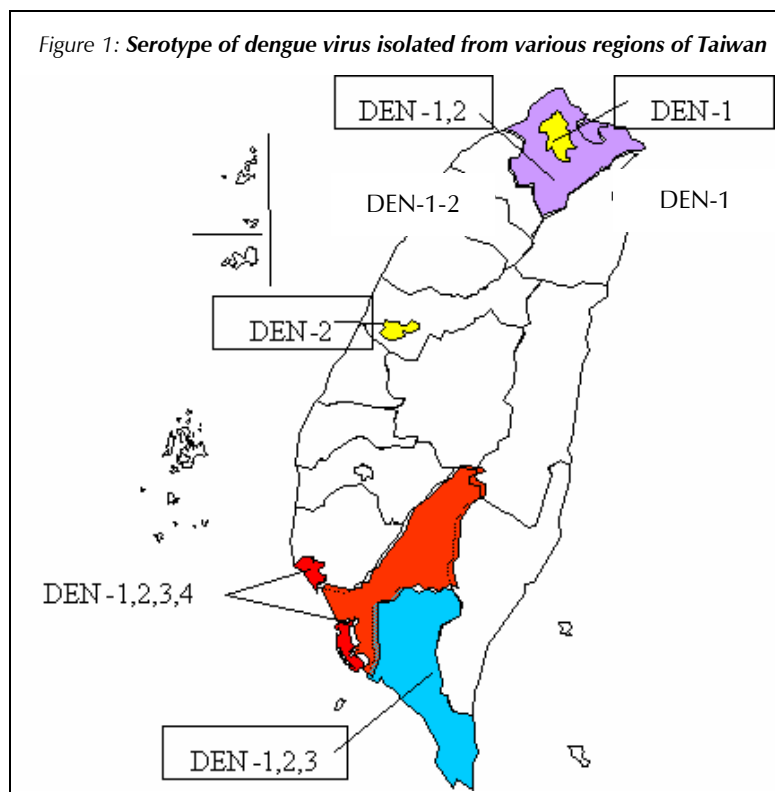
	Region of infection	Imported cases	Serotypes <sup>+</sup>	
			DEN-3	DEN-4
Imported Cases	Philippines	1	1	-
	Indonesia	9	-	-
	Malaysia	3	-	-
	Cambodia	2	-	-
	Laos	1	-	-
	Malaysia or Thailand	1	-	-
	Viet Nam	1	-	-
	Bangladesh	2	-	-
	India	1	-	-
	Costa Rica	1	-	-
	Bangladesh or Indonesia	1	-	-
Indigenous Cases	Residential area	Indigenous cases	Serotypes	
			DEN-3	DEN-4
	Pingtung County	2	1	-
	Tainan City	95	-	10
	Kaohsiung County *	2	-	-
	Taipei County **	3	-	-
Grand total		125	2	10

\* One of the cases was infected in Tainan City

\*\* Two of the cases were infected in Tainan City

+ Typed by RT-PCR or virus isolation

- No virus isolation



## Dengue control programme

A joint dengue prevention and control centre was set up in December 1988 by the Department of Health and the Environmental Protection Administration to plan programmes for the prevention and control of dengue after an outbreak in southern Taiwan. In April 1990, the Executive Yuan approved a five-year plan for the control of dengue fever for implementation. Seven working groups were organized to conduct disease surveillance, laboratory diagnosis, vectors surveillance, insecticide application, source reduction, medical care, and health education to

supervise local governments to carry out control measures. This programme has been extended for another five years and has now become permanent since 2001. These components and their responsibilities are discussed below:

## Surveillance system

To strengthen disease surveillance for dengue fever, the administration set up an active surveillance system throughout the country. In addition to the routine reporting system by physicians, several routes are established to find potential cases: (i) the



public are encouraged to come forward for testing; (ii) health statements of inbound passengers are reviewed; (iii) specimens of patients with unknown fevers are collected for laboratory diagnosis; (iv) schools are asked to report any probable cases; (v) blood specimens are collected from communities; (vi) specimens from other fevers are serologically screened; and (vii) contacts of confirmed cases are investigated for early detection of cases to prevent the spread of the infection.

## Laboratory diagnosis

Every dengue case is confirmed by laboratory diagnosis. All serum specimens collected from suspected cases were tested by capture IgM and IgG ELISA. Serum specimens collected within 7 days of the onset were also tested by RT-PCR and virus isolation. A confirmed dengue case is defined as follows: (i) positive for dengue virus isolation; or (ii) positive for dengue virus genomic sequence by RT-PCR; or (iii) four-fold increase of dengue virus-specific IgM or IgG antibody in paired serum samples where cross-reactions to JE have been excluded; or (iv) positive for dengue virus-specific IgM and IgG in a single serum sample where cross-reactions to JE have been excluded. All these assay kits are developed by the Center of Disease Control, Taiwan. Laboratory results are to be completed within 24 hours for ELISA, 48 hours for RT-PCR and 14 days for virus isolation. The results are faxed to local public health bureaus as soon as possible for further action. Local public health personnel then conduct an epidemiological survey and investigate the potential source of dengue

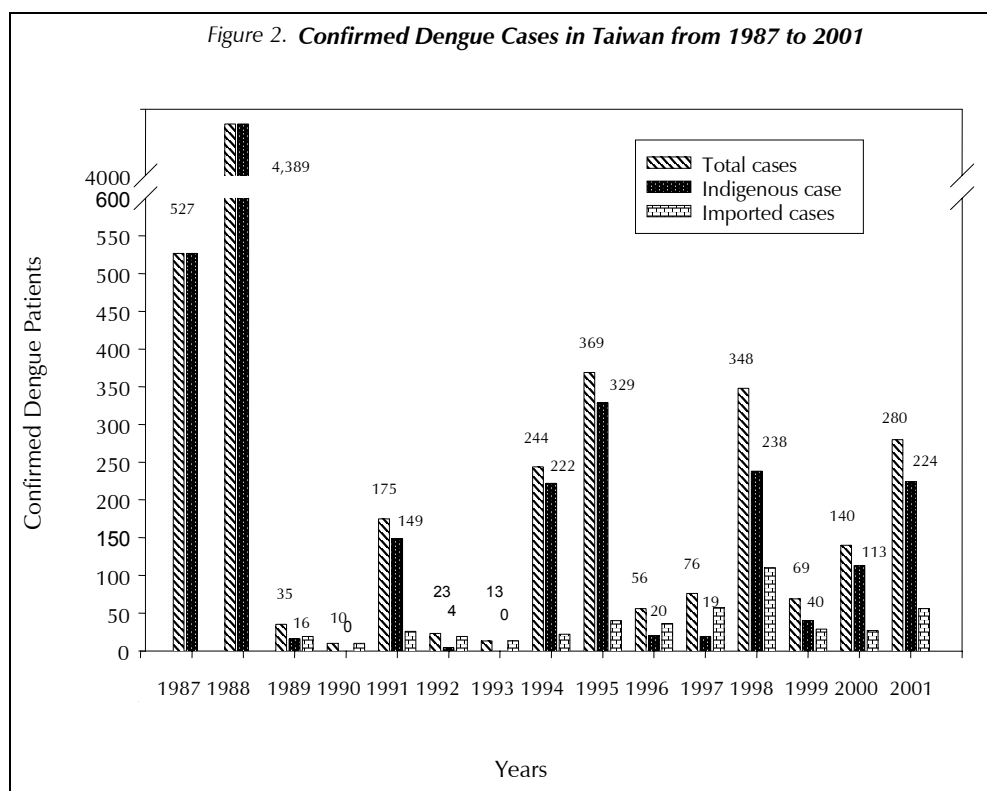
virus introduction. If the patient had travelled abroad in the past two weeks, it will be recognized as an imported case. When travelling abroad has been excluded, it will be recorded as an indigenous case. For an indigenous case, the neighbouring 50 households would be interviewed to determine the possible source of infection. More than 100 blood specimens are mandatory to be collected with free and informed consent by local public health personnel to determine the degree of the virus spread.

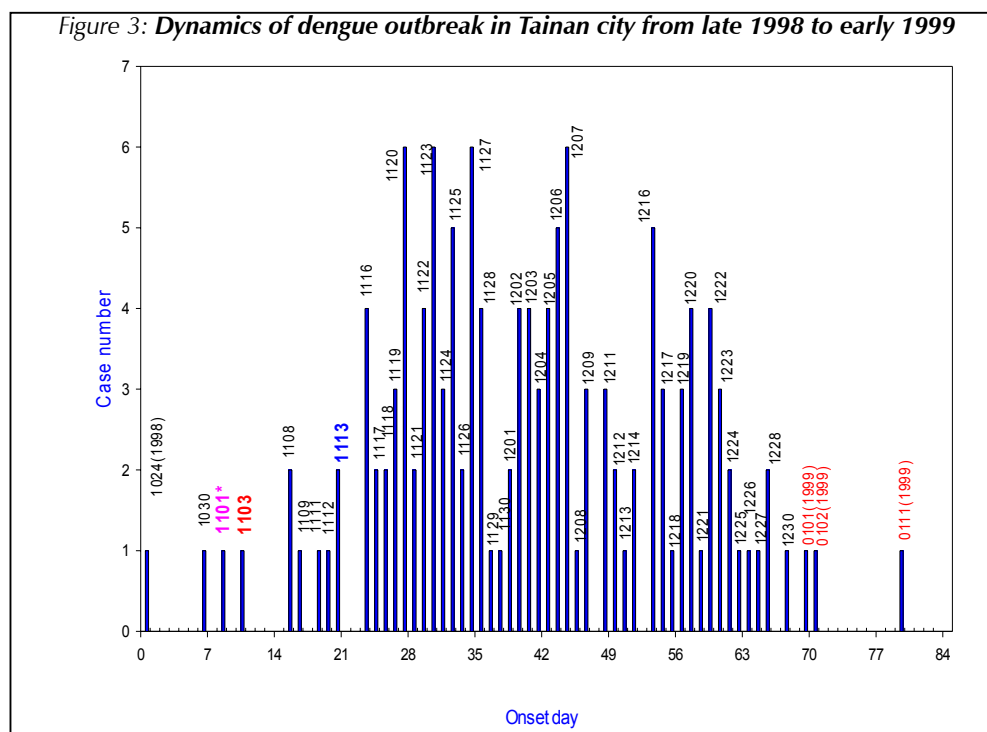
The unique characteristic of dengue transmission in Taiwan is that the importation of the DEN virus occurs through travellers from south-east Asia. Thus, it is very critical to identify the index case of imported dengue patient as quickly as possible, and then break the dengue virus transmission by mosquito vector with insecticides. The success of this approach has been demonstrated from the incidence of dengue cases that occurred in 1987-2001. A large dengue outbreak of 4,389 cases occurred in 1988. After the implementation of the dengue control programme, dengue incidence has been reduced to less than 400 cases per year (Figure 2). However, during the 1998 dengue pandemic, Taiwan Island was also affected. Sporadic cases of both imported and indigenous cases were found. All four dengue serotypes were identified from indigenous cases in Kaohsiung city during August. Among these, a small outbreak with a total of 50 confirmed cases was detected in SenMin district in September. The virus was isolated as DEN-2. The peak of Kaohsiung outbreak was around early November. Tainan city in the north was the next target. The first index case in Tainan city was reported and confirmed on

November 1 (Figure 3). This was identified as an indigenous case because the patient had not travelled abroad in the past two weeks. Active surveillance in the neighbourhood identified two more cases. After epidemiological interview, these two cases were traced back to have had dengue symptoms on October 24 and 30. They both had not travelled abroad, and therefore were both indigenous cases. The virus was isolated as DEN-3, which was distinct from the one that occurred in Kaohsiung city. This suggested that the DEN-3 virus had been circulating in Tainan city for a while. The surveillance system failed to detect it early enough, due probably to the political transition period for the new city mayor and operational failures of local public health

bureaus of the Tainan city government. Dengue virus with *Aedes aegypti* had spread from the sub-district area to the whole city during November and December. The local environmental protection administration undertook vector control activities and brought it under control by late December. The surveillance system has since been improved after this bitter experience in the 1998 outbreak. This example explains the uniqueness of dengue transmission in Taiwan where the major source of dengue virus comes from south-east Asia. The most critical element for dengue control is to identify the index case of imported dengue patient, then break the dengue virus transmission cycle as quickly as possible.

Figure 2. **Confirmed Dengue Cases in Taiwan from 1987 to 2001**





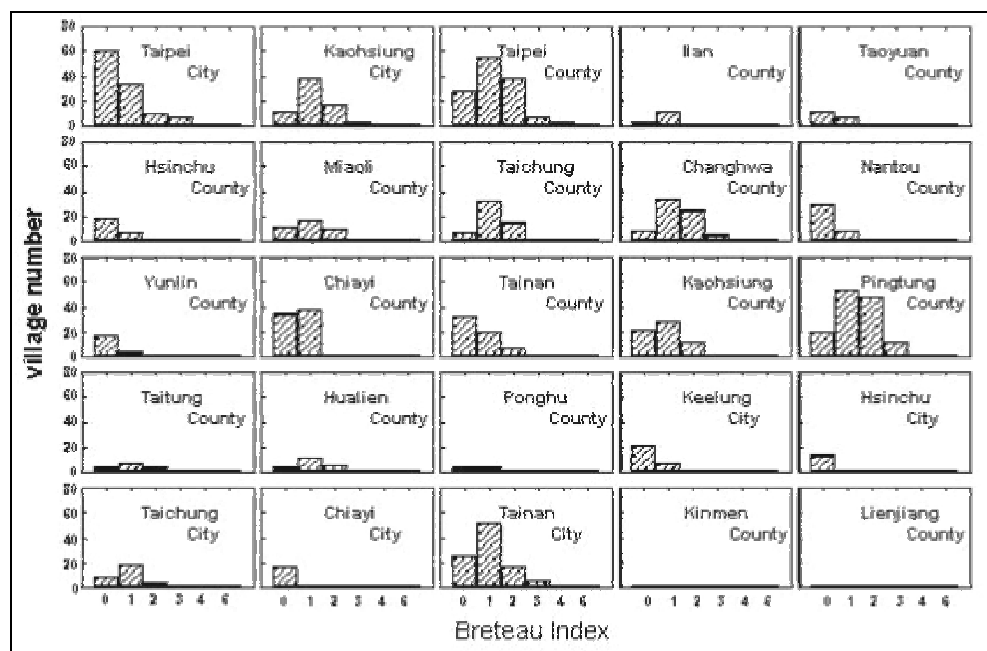
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## Entomological surveillance

In vector surveillance, local public health bureaus routinely conduct mosquito surveys and control measures are enforced if the mosquito density reaches alarming levels. Breteau Index is routinely used for the measurement of mosquito larval density and level three is set to start control measure. The main vectors of dengue in Taiwan are *Aedes aegypti* and *Aedes albopictus*. The distribution of the former species is geographically limited to areas south of Taiwan, and this species is more important in the epidemiology of dengue in southern Taiwan. *Aedes albopictus* is distributed throughout the island, below an elevation of 1,500 m above sea level. This mosquito is

responsible for epidemics of dengue in those areas where *Aedes aegypti* is non-existent, such as the small outbreaks in Chungho, Taipei County; Tung-Hai University, Taichung; and Taipei city. The main breeding containers of dengue vectors in Taiwan are flower vases, water buckets, tyres, bottles, refrigerator receptacles, earthenware jars and concrete tanks. Figure 4 shows the distribution of *Aedes* as measured by Breteau Index by Administrative Area in May 2001. This type of information is released every month to alert the public about the risk of developing dengue fever. An annual training programme, which includes both basic knowledge and field practice, is also conducted for dengue control personnel.

Figure 4: *The distribution of Aedes measured by Breteau Index by administrative area in May 2001*



## Insecticide application and source reduction

Environmental Protection Administration units carry out survey of surrounding areas of confirmed indigenous cases, i.e. houses and work places within 50 metres. All these places will be sprayed with insecticides to reduce the vector source. Fogging was conducted using ULV fogging, both inside and outside the houses. The insecticides used were permissible pyrethroid formulations. The main insecticide contained pyrethrin as the major component including premethrin, alphacypermethrin, cyfluthrin and phenothrin.

## Case management of DHF/DSS

A mission-oriented group "DHF Clinical Consultants Group" led by experienced clinicians has been set up to help evaluate the suspected DHF cases and provide adequate medical care. Clinical diagnosis of DF and DHF/DSS was defined according to the criteria of the World Health Organization<sup>(3)</sup>. However, physicians in Taiwan are generally not familiar with or alert to the symptoms of dengue haemorrhagic fever. There is a need to develop this expertise through an exchange programme of clinicians with centres of excellence in countries of south-east Asia.



imported dengue patient and then break the dengue virus transmission as quickly as possible. This approach has been very effective since 1988. However, the cost is very high and the task is time-consuming. The future goal to resolve dengue virus infection is to understand the pathogenesis

of dengue haemorrhage fever and dengue shock syndrome<sup>(4)</sup> and interrupt the development of the disease process. Alternatively, developing the dengue vaccine is the final answer to prevent dengue virus infection.

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# Time-Series Analysis of Dengue Fever/Dengue Haemorrhagic Fever in Myanmar since 1991

by

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## Abstract

This study assessed the current situation and trends of dengue fever/dengue haemorrhagic fever (DF/DHF) in Myanmar and its states/divisions. It made forecasts from the epidemiological standpoint and also attempted a comparison with Thailand. Between 1991 and 2001, the distribution of DF/DHF showed upward trends with cyclical pattern and seasonal variation. Heterogenous distribution of DF/DHF in Myanmar was evident. According to our model, holding other things constant, the next spike of DF/DHF in Myanmar is to be predicted in 2004 [mean morbidity rate (MBR) = 127.6, 95%; Confidence Interval (CI) = 22.4-232.8]. Computed seasonal index (SI) showed that the transmission of DF/DHF in a given year began in the latter part of the previous year (November and December). Compared to Thailand, between 1991 and 1998, relatively low morbidity with a higher case fatality rate (CFR) was observed in Myanmar.

**Keywords:** Dengue haemorrhagic fever, trend, forecast, seasonality, Myanmar.

## Introduction

Myanmar is located in the tropical zone and the climatic conditions are favourable for the breeding of *Aedes aegypti*, the vector of dengue virus. The first epidemic of dengue fever/dengue haemorrhagic fever (DF/DHF) in Myanmar, pertinent to Yangon city was recorded in 1970. Since then, the disease

has spread to other regions in the country. In Myanmar, DF/DHF is classified as a notifiable infectious disease because of national interest in the development of effective community-based strategies. From 1970 to 2001, records show that an epidemic or outbreak of DF/DHF has occurred every 3 to 4 years<sup>(1)</sup>. In a recent study, Cho-Min-Naing<sup>(2)</sup> has estimated an

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annual loss of 3,269 future life years due to premature mortality and disability during the period 1970-1997. This had a significant impact on national development through the loss of potential learning and earning capacities.

In the context of the national health plan, the Myanmar National DF/DHF Control Project (NDCP) is one of the core elements of the National Vector-Borne Disease Control programme (VBDC). The general objective of the Myanmar NDCP is to prevent the occurrence of cases and to reduce deaths due to DF/DHF. Despite concerted efforts for the prevention and control of DF/DHF, the increasing trend of the disease highlights the need for better planning for effective and efficient control strategies and trend analysis studies. Most studies of DF/DHF in Myanmar have attempted a descriptive summarization using aggregate data<sup>(3,4)</sup>. Previous studies employed simple linear regression to obtain trends in absolute numbers of cases and deaths. Data such as morbidity rate (MBR), mortality rate (MTR) and case fatality ratio (CFR) are comparable and thus more informative, while absolute numbers are not directly comparable. Moreover, most infectious diseases exhibit temporal variation characterized by seasonal and cyclical variations, social trends and epidemics. Therefore, a model considering contributory components to time-series analysis such as seasonal components, cyclical patterns and error terms is valuable. In addition, random or stochastic properties of DF/DHF are important in times series data. If time-series data are non-stationary but integrated as in the present DF/DHF time series analysis, forecasting with Autoregressive Integrated

Moving Average (ARIMA) modelling is attractive<sup>(5)</sup>.

To our knowledge, systematic investigation of seasonal transmission indicating the seasonal index (SI) of DF/DHF is essential in Myanmar. Furthermore, separate analysis of area-wise distribution in each state/division is desirable because of focal distribution of the disease. In addition, studies incorporating comparison with neighbouring countries are still limited. Therefore the following studies were designed:

- (a) to monitor the DF/DHF situation in Myanmar and forecast with ARIMA modelling;
- (b) to detect seasonal transmission patterns in the distribution of DF/DHF in Myanmar, and
- (c) to compare with Thailand and explore possible reasons for the differences or similarities.

## **Methods and materials**

The present study is a time-series analysis using information obtained from official documents and published data. National data on the annual mean cases and deaths, the annual mean MBR and MTR, the annual mean age-specific cases and deaths, and the annual mean CFR for the period 1991-2001 were extracted from official annual reports of the Myanmar VBDC. The aggregate monthly cases and deaths of DF/DHF in Myanmar during 1996-2001 were collected from the same source. The annual mean morbidity and mortality rates for Thailand between 1991-1998 were obtained from the published data available at the time of



analysis<sup>(6,7)</sup>. As for Myanmar as a whole, the yearly and monthly distribution and age-wise distribution of DF/DHF, descriptive statistics using mean and 95% confidence intervals (CI) for means were applied. To investigate the trend and for forecasting of DF/DHF incidences in Myanmar, time-series analysis pertinent to ARIMA modelling was carried out. In this ARIMA process appropriate autoregressive (AR), differences (D) and moving average terms (MA) were defined based on model diagnostic tests. Diagnostic tests with residuals of autocorrelation function (ACF) and partial autocorrelation function (PCF) were checked for the appropriateness of selected parameters<sup>(5)</sup>.

In order to detect geographical variation, the weighted mean MBR, weighted mean MTR and weighted mean CFR of each of the 14 states/divisions were tested with analysis of variance (ANOVA). The weighted means were calculated as exponential averages with more weight being given to recent observations as compared to observations further in the past. After trials of several arbitrary weights, a weight of 0.7 was selected. The weights thus given to each year's data were 34% to 2001, 24% to 2000, 17% to 1999, 11.5% to 1998, 8.1% to 1997 and 5.5% to 1996. Hypothesis testing was carried out thus:

$H_0$ : The mean of DF/DHF is equal in the 14 groups (states/divisions) of Myanmar

$H_A$ : The means are not equal.

To estimate the seasonal transmission, an SI was computed using reported monthly cases whose base is the MA centred at that month. In the present analysis, a moving time length of 6 months was considered as a moving window to detect the bimonthly

pattern of dengue transmission. SI indicates the typical effect of any particular month in the time series. Specifically, we selected a multiplicative form of decomposition of the time-series data to obtain a multiplicative relationship among the components of the data such as trend component, seasonal component, cyclical component and random component. For interpretation, an index of 1.0 in a particular month means that the number of cases in that month is 100% of those in an average month<sup>(8)</sup>.

Based on available data for 1991-1998, for comparison of the incidences of DF/DHF between Myanmar and a neighbouring country (Thailand in our case), a matched-group t test was applied with an  $\alpha$  error of 0.05. In this aspect, our hypothesis was illustrated as:

$H_0$ :  $\mu_1 = \mu_2$  [The mean morbidity rate/mortality rate of DF/DHF is equal in the two countries]

$H_A$ :  $\mu_1 \neq \mu_2$  [The means are not equal]

The data in the present study were processed using Excel spreadsheet and Minitab software (release 11.2) for Windows. All significance tests were two-sided.

## Results

Table 1 shows the distribution of national MBR, MTR and CFR of DF/DHF in Myanmar between 1991-2001. It appears that during this period, the yearly averages of DF/DHF in Myanmar were significantly different at the 5% level ( $p < 0.05$  for all three variables).

**Table 1:** Distribution of yearly means of DF/DHF in Myanmar (1991-2001)

Description	Mean* (95%CI)
Cases	6,207 (2,709-9,705)
Deaths	138 (43-233)
Case fatality ratio	2.13 (1.38-2.87)
Morbidity rate per 100,000 under 15 population	35.25 (16.4-54.08)
Mortality rate per 100,000 under 15 population	0.89 (0.34-1.45)

\*Mean for 11 observations (1991-2001); CI denotes confidence interval

On the evidence presented in Figure 1, sharp spikes are clear in the years 1994,

1998 and 2001. It is noticeable that the magnitude of DF/DHF (that is, MBR in the figure) in these spike years reached beyond the upper limit of 95% CI for the mean. Based on the recent past nonepidemic year 2000, our forecasting model indicates that, holding other things constant, the next expected morbidity spike may occur in the year 2004 (with mean MBR = 127.6; 95%, CI = 22.4-232.8). The same pattern occurs with MTR (Figure not shown).

The available data set shows that the highest affected age group was 5-11 years, followed by 1-4 years and 12-14 years (Table 2). The least affected age group was, in general, 15 years and above. Comparing 1998 and 1999, there was no significant difference in age-wise distribution ( $p > 0.05$  in all age groups).

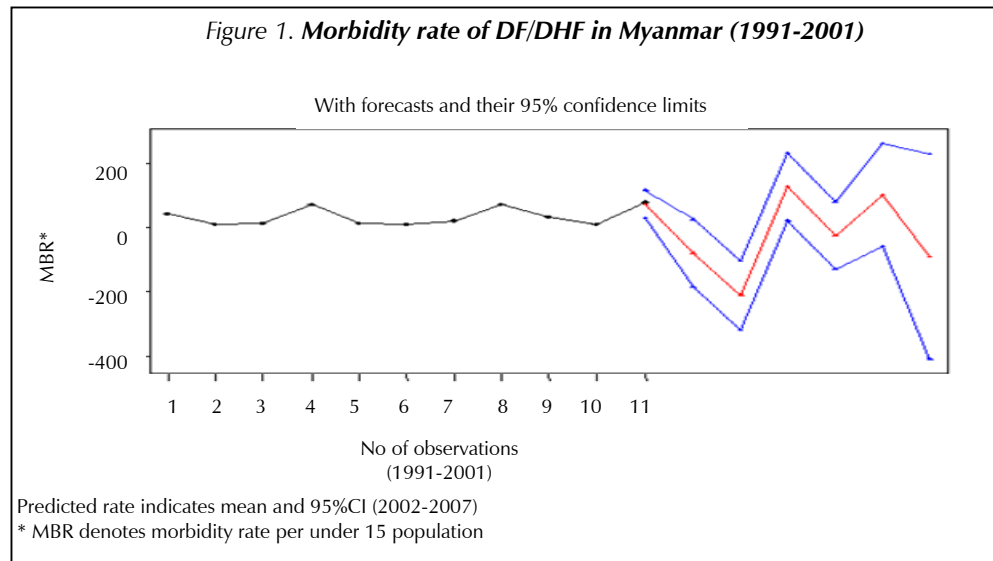


Table 3 illustrates the distribution of DF/DHF among 14 states/divisions. It is noted that until 2001, no cases and deaths were recorded in Chin State of Myanmar. The highest number of cases, deaths and MBR are noted in Yangon division, while the

highest MTR was in Mon state and the highest CFR in Kayah state division during the course of the study period. The distribution of DF/DHF in 14 states/ divisions showed significantly different levels ( $p < 0.001$  in all variables).

**Table 2.** Percentage distribution of age-wise break-up of DF/DHF cases in Myanmar (1998-1999)

Year	<1year	1-4 years	5-11 years	12-14 years	15-20 years	21-24 years	25-30 years	≥31 years
1998	4.38	32.16	52.65	9.91	0.45	0.14	0.12	0.18
1999	5.11	32.82	52.99	8.97	0.1	0	0	0

Note: Age grouping is as it appeared in the office document.

**Table 3:** The weighted mean distribution of DF/DHF in 14 States/Divisions of Myanmar between 1996-2001

State/Division	Weighted cases	Weighted deaths	Weighted MBR	Weighted MTR	Weighted CFR
Kachin	252.35	8.63	21.63	1.82	3.42
Kayah	0.97	0.17	1.175	0.21	17.53
Kayin	279.8	3.37	11.94	0.59	1.2
Chin	0	0	0	0	0
Mon	1263.08	23.33	6.91	2.63	1.85
Rakhine	64.78	2.51	5.789	0.24	3.88
Shan	63.83	3.16	4.51	0.17	4.94
Ayeyarwaddy	386.05	11.76	9.28	0.44	3.05
Magwe	112.21	8.34	2.88	0.47	7.43
Mandalay	856.14	5.87	22.04	0.25	0.69
Bago	577.09	11.59	15.31	0.58	2.01
Yangon	3911.49	28.99	82.3	1.41	0.74
Sagaing	257.63	4.72	6.1	0.26	1.83
Tanintharyi	131.52	2.29	24.38	0.54	1.74

F- test is significant at  $p < 0.0001$  in all 5 variables

MBR denotes morbidity rate per 100,000 under 15-yr population

MTR denotes mortality rate per 100,000 under 15-yr population

CFR denotes case fatality ratio

Figure 2 illustrates the monthly reported cases of DF/DHF in Myanmar between 1996-2001. In Myanmar as a whole, a higher number of cases were reported in the monsoon period with no case-free month being observed during the study period

As  $SI \geq 1$  presumes transmission season, the dengue transmission season in Myanmar generally peaked in the early and latter parts of the year during the period 1996 to 2001

(Table 4). The highest SI was observed in the later two months in 2000, a pre-epidemic year. This suggests that the epidemic in 2001 was due to dengue transmission in November-December of 2000. It then became prolonged and covered the first four months and the last four months of 2001 according to the present finding. Yet, perennial transmission of dengue was not evident during the period 1996-2000. Surveillance system

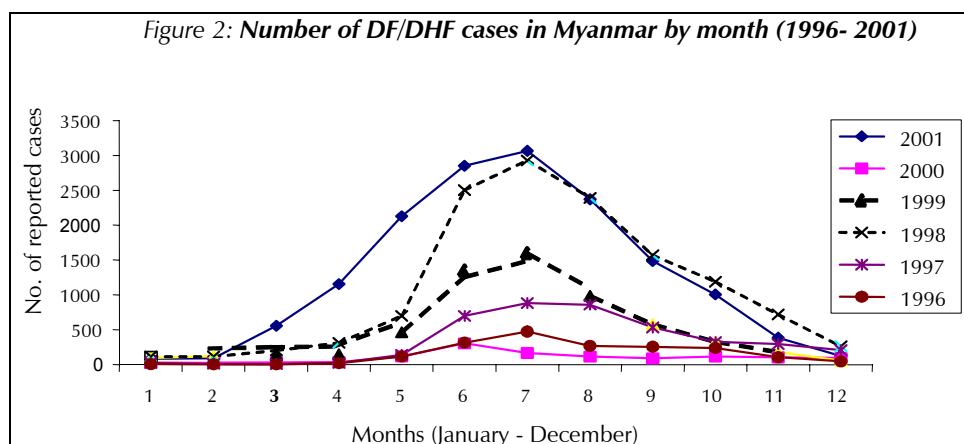


Table 4: Seasonal indices (SI) in Myanmar (1996-2001)

Month	1996	1997	1998 <sup>♦</sup>	1999	2000	2001 <sup>♦</sup>
	SI	SI	SI	SI	SI	SI
January & February	1.77*	1.6*	1.54*	1.66*	1.08*	1.23*
March & April	0.89	1.35*	1.15*	1.08*	0.7	1.01*
May & June	0.88	0.84	0.81	0.73	0.62	0.77
July & August	0.2	0.14	0.38	0.3	0.39	0.74
September & October	0.75	0.46	0.56	0.67	0.97	1.03*
November & December	1.49*	1.59*	1.54*	1.53*	2.22*	1.19*

Moving length = 6 (bimonthly considerations)

\*Aggregate data of 14 States/Divisions in Myanmar

SI > 1.0

♦ epidemic year<sup>(1)</sup>

Based on available data, Table 5 demonstrates the MBR, MTR and CFR in Myanmar and Thailand in 1991-1998.

A noticeable point is the relatively low MBR with higher CFR in Myanmar. The present estimation reveals that there were significant differences in MBR and CFR of DF/DHF between Myanmar and Thailand during the period 1991-1998 ( $p=0.002$ ;  $p=0.001$ ) but not MTR ( $p=0.131$ ).

## Discussion

Compared to previous studies<sup>(3,4)</sup>, our finding also indicates an upward DF/DHF

trend with cyclical pattern and seasonal variation. The estimated next spike of DF/DHF calls for serious preparedness for an epidemic with sound planning of multisectoral efforts, in order to reduce the expected mortality.

The result of this study confirmed the geographical variation among different states/divisions in Myanmar. Mobilization of health resources should be prioritized for use in the focal areas. This does not mean prevention is not necessary for the as yet non-infected regions. Community awareness of dengue is desirable even in the unaffected regions so as to recognize

**Table 5:** Yearly distribution of DF/DHF in Myanmar and Thailand (1991-1998)

Year	Case Fertility Ratio*		Morbidity rate per 100,000 population		Mortality rate per 100,000 population	
	Myanmar	Thailand	Myanmar**	Thailand	Myanmar	Thailand
1991	4.16	0.31	16.34	76.79 <sup>*</sup>	0.68	0.24 <sup>*</sup>
1992	2.2	0.3	3.99	71.16 <sup>*</sup>	0.09	0.24 <sup>*</sup>
1993	2.94	0.33	5.28	114.88 <sup>*</sup>	0.16	0.38 <sup>*</sup>
1994	3.66	0.27	29.63	87.47 <sup>*</sup>	1.08	0.24 <sup>*</sup>
1995	2.14	0.3	5.52	101.46 <sup>*</sup>	0.12	0.31 <sup>*</sup>
1996	0.97	0.31	4.06	63.09 <sup>*</sup>	0.04	0.19 <sup>*</sup>
1997	2.05	0.25	8.61	169.13 <sup>#</sup>	0.18	0.42 <sup>#</sup>
1998	1.62	0.34	27.46	207.75 <sup>#</sup>	0.45	0.71 <sup>#</sup>

\*cases/deaths in the same year

\*\* transformed original value of rate per 100,000 under 15 population<sup>(1)</sup> to rate per 100,000 population.

<sup>\*</sup>Wangroongsarb, 1997<sup>(6)</sup>

<sup>#</sup>Rojanapithayakorn, 1998<sup>(7)</sup>

the disease as early as possible. Since the major victims of DF/DHF in Myanmar are schoolgoing children, their learning capacity is affected as a result of absenteeism along with post-infection weakness. Moreover, the intangible costs related to anxiety among affected community members have to be compounded.

As indicated above, the transmission of DF/DHF in a given year begins in the latter part of the previous year (November and December). This finding has implications for the timing of (vector breeding) source reduction efforts. If such efforts are started even during the early monsoon as is usual in Myanmar, it is already late.

If we suppose data quality to be the same for Myanmar and Thailand, the relatively low morbidity with higher CFR in Myanmar raises two possibilities. First, if this is really a higher CFR, it may be due to late admissions to hospitals for a variety of reasons, which, in turn, leads to serious complications such as dengue shock syndrome, contributing to the high mortality of DHF. Here, our key assumption is that the quality of case management is standardized in both countries. The second explanation rests on the point that there may be a possibility of a low denominator factor, giving exaggerated results due to reporting bias. Because the estimation of CFR in Myanmar has employed only admitted cases (Grade II and above) as Grade I cases are not admitted to hospital<sup>(1)</sup>. Whatever the explanation, this study shows that DF/DHF is a problem in both countries sharing a border, which needs close collaboration in synergistic control strategies for dengue

infection coupled with individual national strengthening of future efforts.

We are aware that this retrospective study has several limitations. First, the majority of cases are, of course, clinically suspected ones. Since serological confirmation is limited, this finding does not reflect the actual magnitude of DF/DHF in Myanmar. While serological surveillance of DF/DHF in Myanmar is still limited, it is possible that over-diagnosis may be offset by the under-reporting of DF/DHF. This is because Grade I cases are not admitted to hospital and thus they are not included in the case report<sup>(1)</sup>, but the extent of underestimation is unknown. We do recognize that laboratory surveillance aiming to identify the circulating viral serotype(s) of DF/DHF in Myanmar remains a desirable research area.

Regarding the methodology, the use of ARIMA modelling in the present study “lets the data speak for themselves”. This is what is called an a-theoretic model<sup>(5)</sup>. If we would like to capture additional explanatory factors, a further model incorporating all possible explanatory factors is desirable. For a better understanding of DF/DHF in Myanmar, an investigation incorporating other parameters including meteorological factors and entomological factors is presently under way.

Despite limitations, the present study provides evidence-based information regarding “where we are now” and “what we have to expect” to those who will utilize our findings as decision variables for better planning of DF/DHF prevention and control in Myanmar.

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# Dengue Patients at the Children's Hospital, Bangkok: 1995-1999 Review

by

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## Abstract

A retrospective review was done of 5,332 patients who were admitted to the Children's Hospital (Inpatient Department-IPD) with the diagnosis of dengue fever (DF)/dengue haemorrhagic fever (DHF) between 1995-1999. During the same period, 17,908 dengue patients were seen at the Outpatient Department (OPD). The OPD:IPD ratio of dengue patients was about 3:1. Dengue viruses were identified in 2,398 cases (50.6%). DEN-3 was the predominant serotype found in about half of the cases. DEN-1, DEN-2 and DEN-4 were found in 25.8%, 20.9% and 2.7% cases respectively. The percentage of admitted patients was recorded as 19% DF, 56.3% DHF and 24.7% dengue shock syndrome (DSS). Most DHF/DSS patients recovered with only crystalloid solutions. Dextran-40 was used in 28.6% and 3% of DSS and DHF patients, while blood transfusion was needed in 12.9% and 5.3% of DSS and DHF patients, respectively. Platelet concentrate was given only in 0.4% of DSS patients. The mean amount of IV fluid given to DHF and DSS patients during critical periods was about moderate dehydration, i.e. 95.2 ml/kg and 56.1 ml/kg and the mean duration of IV fluid was 32.6 hours and 37.6 hours, respectively. Overall clinical diagnosis using the WHO criteria 1997 was confirmed in 96.8% patients (98.1% for DHF and 91.5% for DF).

Compared to a situation about 10 years earlier, the age incidence of dengue patients showed an increasing trend. More DF patients were seen and less DSS patients were found. It is important to note that 55.9% of DSS patients had fever at the time of shock, which was different from what had been learnt that almost all DSS patients were afebrile at the time of shock. There was some increase in the unusual manifestations, i.e. 7% as compared to less than 5% earlier. Encephalopathy accounted in only 0.7% of the cases while associated conditions and infections were found in 2.7% and 3.4% of cases. More patients were found with elevated liver enzyme, AST and ALT, but no increase in the incidence of hepatic failure/encephalopathy. Complication of fluid overload, which could lead to death was found more than before, 4% vs <1%, because of early intravenous fluid administration. The case fatality rate (CFR) reduced from 1.36% to 0.2%.

**Keywords:** Dengue patients, diagnosis, WHO criteria, epidemiological and clinical shift, Children's Hospital, Bangkok.

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## **Introduction**

Dengue infection is the most important of the mosquito-borne human viral diseases. There has been a dramatic increase in the number of cases and the severity of the disease in the past 30 years. Currently some 2.5 billion of the world's population, primarily in tropical developing countries, is at risk of dengue. Tens of millions of cases are estimated to occur each year; hundreds of thousands of these are of the more severe dengue haemorrhagic fever (DHF) that is a leading cause of childhood hospitalization and death in many countries<sup>(1)</sup>.

It was in 1958 when the first outbreak of DHF was recorded in Thailand. Even though the case fatality rate has come down from 13.9% in 1958 to 0.19% in 2000, the morbidity seems to be on the increase<sup>(2)</sup>. The epidemiology and clinical manifestations of dengue in Thailand have been described earlier<sup>(1,3)</sup>. This study aims (i) to assess the WHO criteria for the diagnosis of DF and DHF and to evaluate the fluid management of DHF patients as recommended by WHO; and (ii) to compare variations in the epidemiological and clinical manifestations of DF/DHF/DSS as of today, in comparison to the situation prevailing 10 years ago.

## **Methods and materials**

Hospital records of the DF and DHF patients who were admitted to the Children's Hospital (Queen Sirikit National Institute of Child Health), Bangkok, supported by serological and/or virological confirmation between 1995-1999, were reviewed.\*

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\* Laboratory confirmations were undertaken by the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok.

Serology was done by ELISA and/or haemagglutination inhibition (HI) technique. Polymerase chain reaction (PCR) technique and/or viral isolation were used to identify dengue viruses.

Most of the diagnoses of DF and DHF at the Children's Hospital were based on the WHO clinical criteria<sup>(3)</sup>. The DHF severity was also classified according to the WHO criteria<sup>(4)</sup>.

Clinical and laboratory data relating to DF, DHF and dengue shock syndrome (DSS) cases were analysed using the SPSS programme.

## **Results**

### ***Number of OPD and IPD cases***

There were 17,908 patients diagnosed with dengue infection (DF, DHF or DSS) at the Outpatient Department (OPD) and 5,332 of these were admitted to the Children's Hospital (Inpatient Department-IPD) between 1995-1999. The number of OPD cases ranged from 1,533 in 1995 to the maximum of 5,823 in 1997. The IPD cases ranged from 539 in 1995 to the maximum of 1,479 in 1997. The ratio of OPD:IPD cases ranged from 2.8 to 7.1. About 4.1% were referred patients from both government and private hospitals.

### ***Laboratory confirmation: Serology and virus identification***

The serological and/or virological confirmations for dengue were performed in 4,743 patients (89.0%). There were 22.7% primary infections and 77.3% secondary infections. Primary infection ranged from

20.1% in 1999 to 24.3% in 1997 when the number of dengue cases was the highest. Secondary infection ranged from 75.7% in 1997 to 79.8% in 1999.

In DF patients, 38.5% had primary infection and 61.6% had secondary infection while in DHF/DSS patients, 19.1% had primary infection and 80.9% had secondary infection.

Identification of dengue viruses (1995-1999) was successful in 2,398 patients (50.6%). One dengue isolate could not be identified and one patient had evidence of two concurrently circulating dengue infections (DEN-2 and DEN-1). DEN-3 (50.6%) was the predominant serotype during these years, followed by DEN-1 (25.8%), whose circulation increased continuously from 18.5% in 1995 to 32.5 % in 1999. DEN-2 (20.9%) was the highest in 1995 and recorded a prevalence of 30% and declined continuously to a level of 19.6% in 1999. DEN-4 (2.7%) was the rare serotype all through this study period and its prevalence ranged from 0.5 in 1997 to 8.1% in 1999.

### ***Disease presentations***

Nineteen per cent of the total DF patients in the study period were admitted to the hospital. Admission rates ranged from 14.4% in 1998 to 22.9% in 1996. About 25% of the admitted dengue patients had shock. The percentage of the patients entering shock ranged from 15.3% in 1999 to 27.7% in 1997.

### ***Age and sex***

The mean age for DF cases was observed as 7.9 years, DHF 8 years and DSS 8.1 years. The mean age for DHF and DSS was the same ( $p>0.46$ ). There was a significant increase in the mean age of dengue patients from 7.6 years in 1995-1996 to 8.1-8.2 years in 1997-1999. There was a visible shift in the age groups showing peak incidence. The age group 5-9 years was the most affected by DF. In 1995, 46.25% of the cases were in the age range of 5-9 years and 28.4% in the age range of 10-14 years as compared to 1999 when 41% were in the age range of 5-9 years and 33.2% in the age range 10-14 years.

No gender difference was observed in the DF, DHF or DSS patients.

### ***Signs and symptoms***

Abdominal pain (1995-99) was the most common complaint in dengue patients (38%) – 30.1% for DF, 35.4% for DHF and 49.9% for DSS. History of myalgia in DF, DHF and DSS patients were 12.9%, 9.9% and 8.8%, respectively. Maculopapular rash during the febrile phase was found in 11.6%, 6.7% and 4.9% of DF, DHF and DSS patients, respectively. Typical convalescence rash was found in 19.4%, 19.3% and 18.9% of DF, DHF and DSS patients.

### ***Sensitivity of WHO case definition, 1997***

The clinical presentations and laboratory findings of DF patients are included in Table 1. Tourniquet test was positive in 90.1%, 93.5% and 87.4% of DF, DHF and DSS

patients, respectively. Hepatomegaly was found in 80.4%, 89.9% and 95.5% of DF, DHF and DSS patients, respectively. Shock was found in 24.7% of all admitted dengue patients (44% of all DHF patients). It was noted that of all shock cases, 55.9% were febrile at the time of shock, with temperatures ranging from 38 to 39°C. About 10% of all DHF patients developed shock after admission.

A platelet count of  $\leq 100,000$  cells/cumm. was found in 50.2%, 93.8% and 92.1% of DF, DHF and DSS patients, respectively. The mean platelet count in DF, DHF and DSS patients were 123,599, 63,855 and 53,452 cells/cumm., respectively.

**Table 1:** Results of use of WHO criteria for the diagnosis of DHF at the Children's Hospital during 1995-1999

Clinical Manifestations	DF	DHF	DSS
Fever duration (days)	4.9	5.0	4.8
Positive tourniquet test (%)	90.2	93.6	87.7
Hepatomegaly (%)	80.4	89.9	95.5
Shock (%)	—	—	44.0
Evidence of Plasma leakage			
• Rising HCT $\geq 20\%$	—	42.3	63.8
• Pleural eff %	—	84.2	96.1
Platelet count $\leq 100,000$ cells/cum (%)	50.2	93.8	92.1

— = Nil

Evidence of plasma leakage as shown by 20% rise in haematocrit (Hct) was found in 42.3% and 63.8% of DHF and DSS patients, respectively. Pleural effusion was found in 84.2% and 96.1% of DHF and DSS patients, respectively, who had had chest X-ray done.

Overall clinical diagnoses of DF and DHF, based on the WHO criteria 1997, were confirmed in 96.8% (91.5% for DF and 98.1% for DHF) patients.

## Other laboratory data

### Clinical blood count (CBC)

White blood count WBC  $\leq 5,000$  cells/cumm. was found in 77.7%, 73.2% and 56.1% of DF, DHF and DSS patients, respectively. The mean WBC of DF, DHF and DSS patients was 4,104, 4,347 and 5,541 cells/cumm., respectively.

Atypical lymphocyte (AL) was found in 8%, 9% and 9% in DF, DHF and DSS patients respectively.

In DF, DHF and DSS patients, the findings of tourniquet test positive + WBC  $\leq 5,000$  cells/cumm. were found in 70%, 69.4% and 51.5% , respectively.

### Liver function test (LFT)

The mean aspartate aminotransferase (AST) in DF, DHF and DSS patients was 109 units (U), 192 U and 423 U, respectively. About 95% of dengue patients had elevation of AST. Most of them had mild elevation of AST (78.5% of DF, 70.6% of DHF and 58.7% of DSS patients). Elevation of AST  $>200$  U was found in 9.6% of DF, 25.3% of DHF and 39.2% of DSS patients (Table 2).

The mean alanine aminotransferase (ALT) in DF, DHF and DSS patients was 53 U, 88 U and 159 U, respectively. About 55.3% of dengue patients had elevation of ALT. Most of them had mild elevation of

ALT (31% of DF, 48.4% of DHF and 50.4% of DSS patients). Elevation of ALT >200 U was found in 3.9% of DF, 8.8% of DHF and 16.3% of DSS patients (Table 3).

**Table 2:** AST range as observed during 1995-1999 in DF, DHF and DSS cases

AST Range	DF (%)	DSS (%)	DHF (%)	Total (%)
0-40 Units	100 (11.9)	24 (2.1)	103 (4.1)	227 (5.1)
>40-200 Units	662 (78.5)	656 (58.7)	1,779 (70.6)	3,097 (69.1)
>200 Units	81 (9.6)	438 (39.2)	638 (25.3)	1,157 (25.8)
Total	843	1,118	2,520	4,481

**Table 3:** ALT range observed during 1995-1999 in DF, DHF and DSS cases

ALT RANGE	DF (%)	DHF (%)	DSS (%)	TOTAL (%)
0-40 Units	549 (65)	1,081 (42.8)	372 (33.3)	2,002 (44.6)
>40-200 Units	262 (31.0)	1,221 (48.4)	564 (50.4)	2,047 (45.6)
>200 Units	33 (3.9)	221 (8.8)	182 (16.3)	436 (9.7)
Total	844	2,523	1,118	4,485

The mean albumin (ALB) level in DF, DHF and DSS patients before plasma leakage was 4.5, 4.5, and 4.3 gm%, respectively. The means ALB level of DHF and DSS patients after plasma leakage was 4.1 and 3.6 gm%.

The mean cholesterol (CHOL) level in DF and DHF/DSS patients before plasma leakage was 151, 148, and 145 mg%. The mean ALB level of DHF and DSS patients after plasma leakage was 127 and 112 mg%.

### Coagulogram

There were 38.4%, 64.1% and 73% of DF, DHF and DSS patients, respectively, who had prolonged partial thromboplastin time (PTT), the PTT ratio >1.3.

There were 6.4%, 7.6% and 13.3% of DF, DHF and DSS patients who had prolonged thrombin time (TT), the TT ratio >1.3.

There were 1.3%, 5.2% and 13% of DF, DHF and DSS patients who had prolonged prothrombin time (PT), the international normalizing ratio (INR) >1.3.

### Unusual manifestations

Some unusual manifestations were observed among the study group. These are given in Table 4.

**Table 4:** Unusual manifestations observed during 1995-1999

Associated infections and conditions	1995 (%)	1996 (%)	1997 (%)	1998 (%)	1999 (%)	Total (%)
Encephalopathy	5 (0.9)	5 (0.9)	9 (0.6)	13 (0.9)	3 (0.5)	34 (0.7)
Associated conditions	12 (2.2)	10 (1.8)	51 (3.5)	40 (2.9)	13 (2.1)	126 (2.7)
Associated infections	19 (3.5)	20 (3.6)	44 (3.0)	56 (4.0)	21 (3.4)	158 (3.4)
Total	36 (6.7)	35 (6.3)	104 (7.0)	109 (7.8)	37 (5.9)	318 (6.9)

Encephalopathy was found in 0.7% of all dengue patients. Its occurrence ranged from 0.5% in 1997 and 1999 to 0.9% in 1995, 1996 and 1998.

Associated infections were found in 3.4% of all dengue patients. Its occurrence ranged from 3% in 1997 to 4% in 1998. Most of them were respiratory tract, gastrointestinal and urinary tract infections.

Associated conditions were found in 2.7% of all dengue patients. Their occurrence ranged from 1.8% in 1996 to 3.5% in 1997. Most of them were thalassemia and G-6-PD deficiency.

### Management

Crystalloid solution (5%<sup>\*</sup>DAR)\* was given to 40.1%, 82.7% and 100% of DF, DHF and

DSS patients, respectively. The mean amount of IV fluid given to DF, DHF and DSS patients was 19.1, 56.1 and 95.2 ml/kg, respectively. Moderate dehydration is 88 ml/kg/day.

Dextran-40 (colloid solution) was used in 3% and 28.6% of DHF and DSS. The mean amount of Dextran-40 for DHF and DSS patients was 13 and 18.4 ml/kg, respectively.

Blood transfusion was given to 2.1%, 5.3% and 12.9% of DF, DHF and DSS patients, respectively.

Plasma was given to 0.1% and 4.1% of DHF and DSS patients, respectively.

Platelet concentrate was given to only 0.4% of DSS patients.

\* 5% dextrose in acetated Ringers' solution

The average duration of IV fluid administration was 32.6 and 37.6 hours for DHF and DSS patients.

### **Outcomes**

Fluid overload and early interstitial edema and/or congestive heart failure were found in 4% of all DHF patients. Almost all of these patients were referred patients. Furosimide was given to these patients, some of whom needed ventilatory support.

The average duration in hospital was 3.3, 3.9 and 4.2 days for DF, DHF and DSS patients, respectively.

The case fatality rate (CFR) was 0.2% (DHF patients) and ranged from 0% to 0.3%. The CFR for DSS patients was 0.7%.

### **Discussion**

The number of dengue patient admissions at the Children's Hospital corresponds to the number of the reported cases in the country. In 1997 and 1998 when there were two consecutive large epidemics of dengue, the number of admissions was 1,400-1,500 cases. In non-epidemic years, the number of admissions ranged from 500-600 cases. The ratio between OPD:IPD was about 3:1, except in 1999 when the ratio was 7.1:1, when the National Plan for Prevention and Control of Dengue was established to celebrate the King's 72<sup>nd</sup> birthday and there was an increased awareness among the people of this disease.

Most of the admitted patients had secondary infections (77.3%). Although more than 90% of the DHF patients had secondary infection, the majority did not develop DHF<sup>(5)</sup>. In this study, 61.6% of the patients with secondary infections ended up with DF.

Cases of primary infection usually, but not all, result in DF<sup>(5)</sup>. In this study, 19.1% of the primary infections resulted in DHF.

Tourniquet test was positive in about 90% of DF and DHF patients, as it was repeated every day until defervescence. In DSS, tourniquet was positive in 87.4% cases which was lower than in DF and DHF. This might be due to false negative finding if it was performed during shock. Usually it is not repeated after shock, because the final diagnosis of DSS was already made from the presence of rising Hct and thrombocytopenia. Some experienced clinicians can make clinical diagnosis of DHF from clinical, physical examinations and CBC findings without doing tourniquet test. Tourniquet test is one of the four WHO criteria for making DHF diagnosis. In most DHF cases there was no record of tourniquet test and other bleeding manifestations (petechiae), and DHF could not be diagnosed according to these WHO criteria. For these reasons, WHO criteria for making the diagnosis of DHF needs modification, and it should be based on only two laboratory criteria, i.e. thrombocytopenia and evidence of plasma leakage.

Hepatomegaly was found in 80% of the DF and >90% of the DHF/DSS patients. The percentage of hepatomegaly was rather high as compared to other reports because it was recorded every day until discharge. DHF patients had a tender and larger liver size as compared to DF patients. Usually, the maximum enlargement of the liver happens one day after shock or defervescence.

The finding of tourniquet test positive + WBC  $\leq$  5,000 cells/cumm. was found in 70% of DF patients. This helped in the diagnosis of DF as reported earlier without resorting to the expensive serological test<sup>(7)</sup>.

Platelet  $\leq 100,000$  cells/cumm. was found in one-half of the DF patients and  $>90\%$  of the DHF/DSS patients. The platelet count was observed to be the lowest one day after shock or defervescence. The platelet value correlated very well with the disease severity as reported earlier. DSS had the lowest mean platelet count as compared to DHF and DF patients.

Evidence of plasma leakage was better demonstrated by right lateral decubitus chest X-ray. It was shown in 84.2% and 96.1% of the DHF and DSS cases.

Lowering of the albumin value by 0.4 to 0.7 gm% from the previous value was another significant evidence of plasma leakage in DHF/DSS patients. Single value of albumin is very difficult to be used as an indicator of plasma leakage because the mean value of albumin during leakage was 4.1 gm% which covers the normal range. Single albumin value of  $<3$  gm% may indicate plasma leakage and value between 3-3.5 gm% may suggest plasma leakage in the previously healthy patients.

Lowering of the cholesterol value by 21 to 33 mg% from the previous value was also another significant evidence of plasma leakage in DHF/DSS patients. Single value of cholesterol  $<100$  mg% may indicate plasma leakage and value between 100-120 mg% may suggest plasma leakage in the previously healthy patients.

DF patients should have no coagulation abnormalities but in this study it was found that 38.4%, 6.4% and 1.3% had prolonged PTT, TT and PT in DF, DHF and DSS patients, respectively. The likely explanation was that these patients were actually DHF patients but the diagnosis could not be made because the rise in the Hct was not  $\geq 20\%$

and there was no other evidence of plasma leakage.

The percentage of DSS patients with prolonged PTT, TT and PT was higher than DHF patients. These findings support that there was disseminated intravascular coagulation (DIC) in more than half of the non-shock DHF patients and that shock further enhanced DIC. Prolonged PT was found mostly in patients with profound shock and usually associated with massive bleeding.

Encephalopathy was rare during this five-year period (0.7%). The common causes were hyponatremia, hypocalcemia and hypoglycemia. Hepatic encephalopathy cases were usually associated with prolonged shock and the prognosis was poor as compared to the above-mentioned metabolic causes.

Associated infections and conditions were found in 6% of the dengue patients. The most common associated infections were respiratory tract infections and diarrhoea. *Salmonella* was another common associated infection in dengue patients. The most common associated conditions were thalassemia and G-6-PD deficiency. Even though it was difficult to make the diagnoses in these DHF cases with associated infections and conditions, a careful follow-up and monitoring would definitely show evidence of plasma leakage and thrombocytopenia in every case.

IV fluid is needed in every shock case but it is not necessary to give IV fluid in non-shock cases if the patients could have adequate oral intake. In this study, 18.3% of the admitted DHF cases did not receive IV fluid. Most of the DHF/DSS patients recovered rapidly with the use of crystalloid solutions only. Dextran-40 and blood transfusion were given respectively to 8.7%

and 3.9% of the dengue patients. Those cases that needed blood transfusion usually had prolonged shock or had received aspirin or NSAID as an antipyretic drug. Platelet concentrate was rarely given to DHF/DSS patients (0.1%) at the Children's Hospital. This supports the view that platelet transfusion is not necessary in most cases of DHF/DSS.

The average amount of IV fluid given during the leakage period in non-shock cases was less than maintenance + 5% deficit, and in the DSS cases it was a little

bit higher. This confirmed that the IV fluid estimation given to the DHF patients for the total duration of the plasma leakage was

almost equal to the maintenance + 5% deficit as per the WHO recommendation.

In general, the WHO recommendation for the management of DHF had been used at the Children's Hospital and the results were excellent with the overall confirmation of 96.8%, i.e. 91.5% for DF and 98.1% for DHF. The CFR was 0.2% for the DHF patients and 0.7% for the DSS patients. It should be noted that almost all deaths that occurred here were among the very severe cases referred from other hospitals.

The comparative epidemiological and clinical manifestations of the dengue infection during the study period 1995-1999 and the period from 1982 to 1986 are summarized in Table 5.

**Table 5:** Comparative shift in epidemiological and clinical manifestations of DF/DHF/DSS during 1982-1986 and 1995-1999 at Children's Hospital, Bangkok

Presentations	Elements	1982-1986	1995-1999
<b>Epidemiology</b>	Primary infection in DF	11.3%	38.5%
	Secondary infection in DHF & DSS	95.9%	77.6 - 88.4%
	Serotype		
	DEN 1	6.9%	25.5%
	DEN 2	42.1%	20.9%
	DEN 3	25.5%	50.6%
<b>Clinical and Laboratory findings</b>	DEN 4	25.5%	3.0%
	Mean Age	7.2	8.0
	10-14 yr	22.4%	30.4%
	DF	10.7%	19.0%
	DHF	22.7%	56.4%
	DSS	66.6%	24.7%
	Fever while in shock	<1%	55.9%
	Rising Hct $\geq$ 20%	>90%	42.3-63.6%
	Unusual manifestations	<5%	6.9%
	OT >40 U	80.0%	94.9%
	Fluid overload	<1%	4%
	CFR	1.36	0.2%



More primary infections (38.5%) were found during the study period as compared to 11.3% ten years ago. This may have been due to the change in the circulating dengue serotypes between the two periods. Probably during the study period, susceptible patients had DEN-1 as the primary infection since during 1982-1986, DEN-1 had accounted for only 6.9%.

All dengue serotypes were found circulating during this five-year study period. DEN-3 was the predominant serotype during this period, but it declined from the maximum of 55.5% to 39.8% in 1999. DEN-2 also had a declining trend from the maximum of 30% in 1995 to 19.6% in 1999. In contrast to DEN-3 and DEN-2, DEN-1 increased from 18.5% in 1995 to 32.5% in 1999. DEN-4 was rarely found. The range was from 0.5-8.1% during the past five years.

The percentage of dengue patients in the older age group of 10-14 years increased from 28% to 34%. This corresponded to the finding that the incidence of dengue in patients older than 15 years had increased from 5% to 30% of the total cases in 2001<sup>(2)</sup>. The mean-age incidence had increased from 7.6 years to 8.1-8.2 years. The incidence among infants was the same as before, ranging from 4-6%<sup>(2)</sup>.

About 20% of the patients admitted each year had the milder form of dengue illness. The proportion of more severe DSS admissions was about 25% as compared to 35% in the 1990s<sup>(1)</sup> and 66% in 1986. In 1999, DSS admission was lower (15%) as most patients were brought to the hospital

early when proper IV fluid management was able to prevent shock.

Shock was found in 30.5% of all DHF patients. Classically, DSS patients have no fever while in shock<sup>(1)</sup>. It was very interesting to find that 55.9% of the shock patients in this study still had fever while they developed shock, but the fever was not high, ranging between 38-39°C. This was due to early admission in the hospital and quick administration of IV fluid.

Rising Hct  $\geq$  20% was found only in 42.3% and 63.8% of the DHF and DSS cases while it was reported in  $>90\%$  of the cases between 1982-1986. Most of the DHF/DSS cases had rising Hct levels, between 10-20%. Among these cases, the evidence of plasma leakage was confirmed by chest X-ray or physical findings of pleural effusion and/or ascites. The lesser degree of rising Hct was due to early IV fluid administration, the increased rate of IV fluid when Hct was increasing, and Hct not being done frequently. This posed a problem of DHF diagnosis based on the WHO criteria, for about half of the DHF patients did not have rising Hct  $\geq 20\%$ . Physical findings of pleural effusion and/or ascites should be added to the WHO criteria in the category of evidence of plasma leakage.

Elevation of AST and ALT was found in 94.9% and 55.3% of the dengue patients, which was higher than the previously reported level of 80%<sup>(1,6)</sup>. This could have been due to the presence of different dengue serotypes, particularly DEN-3 which was predominant during this period and was reported to be associated with liver

involvement<sup>(8)</sup>. This elevation of liver enzyme may possibly be related to the increased drug usage or to shock or other factors that would need to be studied further. This abnormal elevation of AST in most suspected cases of dengue infection may be used as an early indicator of dengue illness as suggested earlier<sup>(6)</sup>.

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# Dengue in the Americas

by

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## Abstract

Dengue has been present in the Americas for centuries, but the current situation is dynamic, and is worsening. The main vector, *Aedes aegypti*, infests most countries. Vector control efforts have been largely inadequate. Population growth, especially in urban, low latitude areas, has resulted in a greater risk for contact with a competent vector. An increasing number of cities have reached a size that can support ongoing circulation of dengue viruses. The mobility and increasing connectedness of human populations through rapid international air travel permit regular introductions of the dengue virus via viremic travellers. More geographic areas have concurrent or sequential circulation of more than one serotype, increasing the risk for severe and complicated dengue.

Serological studies document that dengue infections frequently go unrecognized. Other infections, some treatable with specific antibiotics, are often misdiagnosed as dengue fever, and dengue fever is often presumptively diagnosed when other infections are the cause of illness. Rapid, reliable and inexpensive diagnostic tests are unavailable in most areas. Analyses of illness and death related to dengue show that the burden from dengue in tropical and subtropical America is substantial and is often underestimated. The health and economic impact from dengue is similar to that from other infections that have generated much more attention. Study of border areas that share similar geoclimatic conditions (for example, Mexico and the United States) can help identify risk factors and possible protective measures. Well-studied island epidemics (e.g. Cuba) have been valuable in defining the role of sequential infections, duration of relevant immunological memory, and potential impact of genotype in addition to serotype on the clinical course of the disease.

**Keywords:** Dengue, Dengue haemorrhagic fever, epidemiology, vector control, Americas.

## Introduction

Dengue has long been present in the Americas. Historical records suggest that dengue outbreaks may have occurred in the French West Indies and Panama in the

1600s, in Peru, Mexico, Colombia, the United States (Philadelphia) and the Caribbean in the 1700s, and, in addition, in Brazil, Venezuela, and Argentina in the 1800s and the first half of the 1900s<sup>(1)</sup>. Since the 1960s, dengue virus has spread to

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previously unaffected areas in the Americas and threatens a larger population than ever before in the Americas. Circulation of more than one serotype has been documented in more and more regions, increasing the risk of serious and complicated infections, i.e. dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF)<sup>(2,3)</sup>. Trends in the region, including increasing urbanization, increasing travel and migration of humans, and expansion of the areas infested (and reinfested) by competent vectors, suggest that dengue will continue to be an important cause of morbidity and mortality in the region<sup>(4,5,6,7)</sup>. This paper describes recent dengue activity in the Americas and discusses some of the features of its epidemiology and spread in the Western Hemisphere. A description of the 2001-2002 outbreak of dengue in Hawaii, transmitted by *Aedes albopictus*, is not included in this paper. Preliminary reports describing the outbreak in Hawaii are available from the publications of the Centers for Disease Control, Atlanta, and its website ([www.cdc.gov/travel/other/dengue-hawaii.oct2001.htm](http://www.cdc.gov/travel/other/dengue-hawaii.oct2001.htm)).

## History of dengue in the Americas

More than 2.5 billion people, or about 40% of the global population, live in dengue-endemic regions, which encompass tropical and sub-tropical regions worldwide. An estimated 50 to 100 million cases of DF occur each year, including several hundred thousand cases of dengue haemorrhagic fever (DHF)<sup>(4,5)</sup>. Most go unreported. Reports to WHO in 1998 recorded 1.2 million cases of dengue and DHF and 15,000 deaths. Although, globally, the greatest burden from

dengue continues to occur in Asia, the situation in the Americas is dynamic, and is worsening. In 1998, the Americas reported >700,000 cases of DF and >12,000 cases of DHF. This year (2002) Brazil has experienced the worst epidemic in its history (see below). The number of dengue cases in the Americas should be considered in the context of the population size, which is small in comparison to Asia. The population of the Americas (not all of which is located in areas at risk for dengue infection) was estimated at 823 million in 1999, representing less than 14% of the world's population. The population is distributed with about one-third residing in the US, one-third in Mexico and Brazil, and the remainder in the other 45 countries and territories<sup>(8)</sup>.

*Aedes aegypti*, the main vector for dengue in the Americas (note exception in Hawaii, 2001-2002) is believed to have been introduced into the Western Hemisphere on slave ships from West Africa in the fifteenth through the seventeenth centuries. The mosquito may also have reached the New World on European ships from Spain and Portugal. *Aedes aegypti* was well adapted to flourish in water storage containers on ships<sup>(9)</sup>. *Aedes aegypti* became established widely in tropical and temperate areas of the Americas.

An intensive campaign organized by the Pan American Health Organization (PAHO) starting in 1947 to control yellow fever transmission led to eradication of *Ae. aegypti* from all countries in the Americas except the U.S., Suriname, Venezuela and several Caribbean Islands by the late 1960s<sup>(1,4)</sup>. Records show no evidence of epidemic dengue in the Americas from 1946

through 1963, presumably reflecting in part the benefits from the eradication programme. In areas where *Aedes aegypti* was eliminated, transmission of dengue virus was interrupted. Support for programmes waned and vector control activities declined allowing the mosquito to reinfest areas where it had been eliminated and to spread to areas where it had never previously been recorded. *Aedes aegypti* was repeatedly introduced from areas where the vector was not controlled. Dengue re-emerged in the 1960s and 1970s, initially affecting Jamaica, then Puerto Rico, other Caribbean islands, and Venezuela<sup>(10,11,12)</sup>. Subsequently, dengue

was reported from at least 43 countries in the region<sup>(1,4,5,6,7,10)</sup>. Outbreaks of classic dengue appeared, but these were followed by the appearance of DHF, initially in a massive epidemic in Cuba in 1981<sup>(10)</sup> and since then in other parts of the Caribbean and Central and South America<sup>(13)</sup>. The second major DHF epidemic occurred in 1989-1990 in Venezuela, when 3,108 cases of DHF were identified<sup>(14)</sup>. Table 1 provides a chronological listing of the country locations where different dengue serotypes have been documented, starting in 1953.

**Table 1.** The introduction of dengue serotypes (DEN-1, 2, 3, 4) in the Americas: predominant serotypes, arrival of new strains, countries in which they were identified and associated

Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1953		Trinidad			First-ever isolation of DEN-2 in the Americas; no outbreak was reported <sup>(iii)</sup> .
1963-64			Jamaica Puerto Rico Lesser Antilles Venezuela		A Caribbean pandemic occurred <sup>(iv)</sup> . > 27,000 cases occurred in Puerto Rico <sup>(v)</sup> .
1968-69		Caribbean Venezuela	Caribbean Venezuela		DEN-2 and DEN-3 were found in Jamaica <sup>(vi)</sup> .
1971-72		Colombia			<i>Aedes aegypti</i> reinfested the country, followed by a dengue epidemic <sup>(vii)</sup> .
1975-77			Colombia		A second dengue epidemic occurred in Colombia <sup>(vii)</sup> .
1977	Jamaica Cuba				<b>DEN-1 was introduced, and spread in Caribbean, South America, Central America, and led to a pandemic<sup>(viii)</sup>.</b>
1978	Venezuela Colombia Guyana Suriname French Guiana Honduras El Salvador Guatemala Belize Mexico				From 1977-80, 702,000 cases were reported to PAHO from the Americas, but estimates from Colombia, Cuba, and Venezuela suggested >5 million cases <sup>(ix)</sup> . Epidemic in Central America affected Honduras <sup>(x)</sup> , followed by El Salvador, Guatemala, Belize, and spread to Mexico.

Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1980	Texas, USA				Most cases occurred near the Texas-Mexico border <sup>(xi)</sup> .
1981		Cuba		Caribbean S. America C. America Mexico	New DEN-2 strain was introduced in Cuba; <b>1<sup>st</sup> major DHF epidemic</b> , total of 344,203 cases, 10,312 severe, 158 fatal <sup>(xii)</sup> . DEN-4 was introduced <sup>(xiii)</sup> , affecting the Caribbean, northern South America, Central America, and Mexico.  8350 cases <sup>(i)</sup>
1982	Colombia Brazil			Colombia Brazil El Salvador Puerto Rico	6776 cases <sup>(i)</sup> > 12,000 cases <sup>(i)</sup> 5166 cases <sup>(i)</sup> 9536 <sup>(1)</sup>
1983	Colombia  Mexico	Jamaica Trinidad Mexico		Colombia El Salvador Jamaica Trinidad	4977 cases <sup>(xiv)</sup> 3814 cases <sup>(i)</sup> DEN-2 predominated <sup>(xiv)</sup> DEN-4 predominated <sup>(xiv)</sup> 19,028 cases <sup>(i)</sup>
1984					DEN-1,2,4 continued to circulate, causing 43,435 cases in the region <sup>(xv)</sup> .
1985	Nicaragua Aruba Honduras El Salvador Venezuela Colombia Puerto Rico	Nicaragua  Venezuela Colombia Puerto Rico		Venezuela	17,483 cases <sup>(xv,xvi)</sup> 24,000 cases <sup>(i)</sup>     7797 cases <sup>(xv)</sup>
1986	Brazil  Puerto Rico	  Puerto Rico		  Puerto Rico	47,370 cases; major outbreak occurred in Rio de Janeiro <sup>(xvii,xviii)</sup> .  10,659 cases, predominantly DEN-4 <sup>(xix)</sup> , followed by continued outbreaks ever since.
1987	<b>Bolivia</b>				1994 cases and 4847 cases the following year <sup>(i)</sup> .
1988	Paraguay  Ecuador				405 cases followed by 41,800 cases the following year <sup>(i)</sup> . Epidemic occurred in Guayaquil, following <i>Aedes aegypti</i> reinfestation (detected in 1985) <sup>(xx)</sup> .
1989-90	Venezuela	Venezuela		Venezuela	<b>2<sup>nd</sup> DHF epidemic in the Americas</b> – 3108 cases of DHF, 73 deaths, with annual epidemics ever since; DEN-2 was predominant <sup>(xxi)</sup> .
1990	Peru  Brazil	  Brazil		Peru	<b>7858 cases, followed by continued transmission<sup>(i,xxii)</sup>.</b> <i>Epidemic occurred in the state Rio de Janeiro<sup>(xiii)</sup>.</i>

Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1991-1992		French Guiana			DEN-2 predominated although DEN-1 was also present <sup>(xiv)</sup> .
1993	Costa Rica	<b>Panama</b>			1 <sup>st</sup> outbreak since 1942 <sup>(xv)</sup> 4612 cases <sup>(xvi)</sup> .
1994		Brazil Puerto Rico			54,453 cases, reaching 530,578 cases by 1998 <sup>(i,xvii)</sup> . 22,000 cases <sup>(i)</sup> .
	Panama Costa Rica		Nicaragua Panama Costa Rica		Reappearance of DEN-3; total 20,469 cases; 1247 cases DHF; incidence 4.8/1000 <sup>(xv)</sup> . DEN-3 was of the Sri Lanka/India genotype, DEN-1 was predominant <sup>(xv)</sup> . 13,929 cases <sup>(i)</sup>
1995			Mexico Guatemala		DEN-3 identified in Mexico <sup>(xviii)</sup> . Total of 316,411 cases occurred in the Americas <sup>(i)</sup> . DEN-3 predominated for next 3 years <sup>(xix)</sup> .
1996-1997	Brazil	Brazil			Epidemic in Belem, Para State <sup>(xx)</sup> .
1997		Cuba Argentina			DEN-2 re-emerged in Cuba; 2946 laboratory-confirmed cases; 205 DHF/DSS <sup>(xxxi)</sup> . 1 <sup>st</sup> cases since 1916 were identified <sup>(xxxii)</sup> .
1998		Nicaragua	Nicaragua		DEN-3 predominated <sup>(xxxiii)</sup> .
2000	Costa Rica	El Salvador Guatemala Honduras Nicaragua	Nicaragua Costa Rica Brazil		<b>All serotypes are present in the 1990s<sup>(xxxiv)</sup>.</b> <b>16,355 cases</b> <b>5963 cases</b> <b>8715 cases</b> <b>5233 cases</b> <b>2113 cases</b> DEN-3 appeared in Brazil in 2000 <sup>(xxxv)</sup> .
2002	Colombia El Salvador	Colombia El Salvador	Brazil Colombia El Salvador	Colombia El Salvador	Huge epidemic; 711,919 cases reported, 2229 DHF, 130 deaths as of September 15 <sup>(xxxvi,xxxvii)</sup> . DEN-1, 2, 3, 4 are reported to be circulating in Colombia and El Salvador <sup>(xxxviii)</sup> . Epidemics have also been reported from Honduras, Nicaragua, Venezuela <sup>(xxxviii)</sup> .

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Programmes to control vectors face new challenges. Resistance of vectors to insecticides is increasing, limiting the available options. Resistance of *Aedes aegypti* to pyrethroids is now widespread<sup>(15)</sup>. Other factors have also contributed to increased vector abundance, among them expansion of water storage locations and a plethora of vector breeding sites in used tyres, flower pots, and discarded plastic cups and other non-biodegradable containers. Inadequate piped water supplies lead people to store water in and around homes.

## Human population

The human population has also grown but more relevant is the location of the population expansion. Most of the population growth has occurred in urban areas. Globally, more people now live in urban areas than ever before. In South America, 78% of the population lived in urban areas in 1995; this is projected to increase to 88% by 2025. (The comparable figures for the world are 45% in 1995 and 60% by 2025.) Much of the growth of cities is unplanned and unregulated, and huge slums surround some cities. Many of these areas are characterized by poor housing,

crowded living conditions, inadequate waste disposal and lack of clean water. This contemporary landscape provides an ideal environment for breeding of *Aedes aegypti*, a mosquito that is well adapted to the urban habitat. Poor housing and absence of screens increase the potential contact between mosquitoes and humans. More urban centres have reached a population size that it takes to sustain the ongoing circulation of a dengue virus in a population, which increases the risk of severe forms of dengue.

Recent studies also point to another reason to be concerned about population size. Over the past two centuries the number of dengue lineages has been increasing roughly in parallel with the size of the human population<sup>(16)</sup>. Scientists have suggested that a larger human population in which the virus replicates allows increased opportunities for viral evolution and enhances the potential for the appearance of more virulent strains. Intra-serotype recombination events may also play an important role in genetic diversity of the dengue virus<sup>(17)</sup>. More opportunities for such genetic exchange exist in populations with hyperendemic dengue.

This large and growing urban population in low latitude areas is increasingly linked by air travel to other areas of the world. Global travel continues to increase. In 1996, 2.5 billion persons passed through airports and more than 500 million persons crossed international borders on commercial airplane flights. Air traffic volume has increased about 7% per year for the past 20 years. As of the mid-1990s, about 5000 airports had scheduled worldwide service. This massive movement of the human population links all major urban areas of the world. Speed of travel means that a person bitten by an infective mosquito (carrying dengue) in Thailand can reach home in Brazil before the symptoms of the infection begin to show. Viremia, which may persist for as long as 7-8 days, typically begins 2 days before the onset of the symptoms, during which the viremic human may continue his usual activities and may encounter competent vectors at work, home and elsewhere. Levels of virus in blood may be high. In a study of plasma viral RNA levels in children in Thailand, levels ranged as high as  $10^9$  RNA copies/ml, peaked an average of 2 days before defervescence, and dropped rapidly during the last several days of fever<sup>(18)</sup>. Table 2a contains the tourist arrivals to a number of the countries in the Americas that have had the highest volumes of visits, as well as the recent incidence of dengue in the local population as reported to the Pan American Health Organization (PAHO). Costa Rica reported the population at risk, and the incidence was calculated based on that figure. Bolivia reported the population at risk for 2001 also. The other countries in the table reported projected populations. Table 2b lists international arrivals in each

sub-region. These figures indicate that dengue-endemic countries in the Americas receive over 50 million international arrivals each year.

**Table 2a:** International arrivals in some dengue-endemic countries in the Americas

Country	Inter-national arrivals in 2000 <sup>a</sup>	Incidence of dengue in local population (per 100,000) <sup>b</sup>	
		2000	2001
Argentina	2,949,000	4.59	0.03
Bahamas	1,577,000	0	
Brazil	5,313,000	136.07	239.38
Costa Rica	1,088,000	434.63 <sup>c</sup>	818.163 <sup>c</sup>
Cuba (air arrivals only)	1,741,000	1.23	101.58
Dominican Republic (air arrivals only)	2,972,000	40.75	42.28
Jamaica (air arrivals only)	1,323,000	0.97	1.50
Mexico	20,641,000	21.96	6.19
Peru	1,027,000	21.38	89.41
Puerto Rico (air arrivals only)	3,341,000	62.88	132.41

<sup>a</sup>From World Tourism Organization News Release, [www.world-tourism.org/newsroom/releases/more\\_releases/january2002/numbers\\_2001.htm](http://www.world-tourism.org/newsroom/releases/more_releases/january2002/numbers_2001.htm)

<sup>b</sup>PAHO. [www.paho.org/English/HCP/HCT/VBD/dengue-cases-2000.htm](http://www.paho.org/English/HCP/HCT/VBD/dengue-cases-2000.htm) and [www.paho.org/English/HCP/HCT/VBD/dengue-cases-2001.htm](http://www.paho.org/English/HCP/HCT/VBD/dengue-cases-2001.htm).

<sup>c</sup>Based on population at risk.

**Table 2b:** International arrivals in sub-regions in the Americas in 2000

Sub-Region	Arrivals in 2000 (millions) <sup>a</sup>
North America	91.2 (Canada 19.7; USA 50.9)
Caribbean	17.3
Central America	4.4
South America	15.5 (Chile 1.7; Uruguay 2.0)
Total for Americas	128.3

<sup>a</sup>From World Tourism Organization News Release, [www.world-tourism.org/newsroom/releases/more\\_releases/january2002/numbers\\_2001.htm](http://www.world-tourism.org/newsroom/releases/more_releases/january2002/numbers_2001.htm)

## Vector populations

Shipping and trade have allowed the introduction of *Aedes albopictus*, also a competent vector for dengue, into previously uninfested countries. In 1985, *Aedes albopictus* was introduced into North America, probably via used tyres shipped from north Asia. It was first found in Texas and within 12 years had spread to 678 counties in 25 states<sup>(19,20)</sup>. Its dispersal followed inter-state highways, and presumably was carried with human traffic and trade. As of the late 1990s, *Aedes albopictus* had been introduced into Brazil, the Dominican Republic, Guatemala, Mexico, Cuba, Bolivia, El Salvador and Colombia, and was continuing to spread<sup>(21)</sup>. Modern ships, especially those with container vessels, are effective in dispersing the immature stages of mosquitoes. Most successful mosquito invasions have resulted from ship transport. Desiccation-resistant *Aedes* eggs are effectively transported in tyres. The reinfestation of *Aedes aegypti* and the establishment of *Aedes albopictus* in the Americas provide the vehicles for major

epidemics of dengue fever and dengue haemorrhagic fever in this region.

## Recent dengue outbreaks in specific countries

All four serotypes have circulated widely and continue to cause dengue outbreaks in the Americas<sup>(22)</sup>. All four serotypes have been associated with epidemics in the 1990s in Costa Rica, El Salvador, Guatemala, Honduras and Nicaragua. As of early 1999 all four serotypes had also been documented in Mexico and Puerto Rico<sup>(23,24)</sup>. As of September 15, 2002, numerous countries have reported dengue and DHF to PAHO, including Brazil, Colombia, Costa Rica, Cuba, El Salvador, Honduras, Peru, Venezuela, Barbados, and Trinidad & Tobago<sup>(25)</sup> (See Table 3).

In Brazil, more than 500,000 dengue cases were reported in 1998 and *Aedes aegypti* infested all important urban centres of the country. DEN-1 has been present since 1986 and DEN-2 since 1990<sup>(26,27)</sup>, setting the stage for widespread DHF/DSS if new serotypes of dengue are introduced. As of September 2002, more than 711,919 cases of dengue (predominantly DEN-3, although DEN-1 and DEN-2 are also present) as well as 2,229 cases of DHF and 130 deaths had been reported in the country<sup>(25)</sup>. The dengue epidemic affected Rio de Janeiro state most severely<sup>(28)</sup>.

*Aedes aegypti* reinfested Argentina as far south as Buenos Aires after the vector had been eradicated in 1963. In 1996 and 1997, high levels of infestation were noted in Buenos Aires province and the Federal District. In 1997, viremic travellers brought dengue virus to Argentina and local transmission occurred in

Salta province in northern Argentina. This was the southern most extension of dengue noted to that point<sup>(29)</sup>.

Similarly, Easter Island, Chile and Hawaii (USA) recently suffered outbreaks of dengue. Both outbreaks were caused by DEN-1, and probably had been spread by travellers from Tahiti, the American Samoa or Western Samoa. More than 100 cases of dengue have been reported from Hawaii since 2001<sup>(30)</sup> and more than 130 cases have been reported from Easter Island in 2002<sup>(25)</sup>. Although small in comparison to the outbreaks elsewhere in the Americas, these

mark new territories of dengue invasion in 2001-2002.

Table 3 lists dengue cases by country from 1997 to 2002. Note that Brazil, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Peru, Puerto Rico and Venezuela have had major dengue outbreaks or epidemics during this period. Over 320,000 cases of dengue fever were reported annually from the region since 1997. In the first quarter of 2002, there were already more than 400,000 cases reported.

**Table 3: Countries in the Americas and reported dengue cases (1997-2002)**

Country or Region	1997 <sup>1</sup>	1998 <sup>2</sup>	1999 <sup>3</sup>	2000 <sup>4</sup>	2001 <sup>5</sup>	2002 (as of September 15) <sup>6</sup>
Anguilla	0	0	12	3	25	10
Antigua& Barbuda	7	4	2	8	20	14
Argentina		822	3	1,700	11	234
Aruba			130	76	0	28
Bahamas	0	336	0	0	0	0
Barbados	199	852	696	744	1,043	488
Belize	141	8	3	4	3	19
Bermuda	0	0	3	0	0	0
Bolivia	539	49	43	73	176	661
Bonaire	—	—	—	—	—	—
Brazil	254,109	535,388	204,201	231,471	413,067	711,919
British Virgin Islands	0	1	0	3	23	7
Cayman Islands	0	2	1	0	0	1
Chile (Easter Island)	—	—	—	—	—	636
Colombia	24,290	63,182	20,336	22,775	55,437	34,559
Costa Rica	14,267	2,628	6,040	4,907	9,237	5,509
Cuba	3,012	—	0	138	11,432	3,011
Curacao	—	7	5	10	0	—
Dominica	0	1	1	15	5	0
Dominican Republic	608	3,049	1,088	3,462	3,592	736
Ecuador	3,871	4,606	2,901	22,937	10,919	5,833

Country or Region	1997 <sup>1</sup>	1998 <sup>2</sup>	1999 <sup>3</sup>	2000 <sup>4</sup>	2001 <sup>5</sup>	2002 (as of September 15) <sup>6</sup>
El Salvador	423	1688	626	3248	1093	17,123
French Guiana	851	534	88	186	2830	
Grenada	22	4	85	27	12	221
Guadeloupe	0		41	60	0	
Guatemala	5,385	4,655	3,617	9,006	4,516	3,986
Guyana	0	42	0	19	60	9,180
Haiti	—	—	—	—	—	—
Honduras	11,873	22,218	17,835	13,642	9,077	27,113
Jamaica	16	1,551	24	25	39	27
Martinique	235	44	268	171	4,471	
Mexico	53,541	23,639	14,875	21,715	6210	3005
<b>Montserrat</b>	—	0	0	9	1	4
Netherland Antilles	—	—	—	—	—	—
Nicaragua	3,126	13,592	11,150	7,317	2,104	4,258
Panama	2,628	2,717	2,783	317	1,545	405
Paraguay			1,164	24,282	38	1,795
Peru	1,357	988	554	5,486	23,329	7,196
Puerto Rico	6,955	17,241	4,993	2,433	5,233	1,662
St. Kitts/Nevis	0		1	5	89	18
St. Lucia	12	1	3	0	292	44
St. Martin	—	—	—	—	—	—
St. Vincent & Grenadines	3	88	8	5	3	124
Suriname	90	1,151	695	1,073	760	220
Trinidad & Tobago	784	3,120	1,265	2,066	2,244	2,625
Turks & Caicos Islands	0	—	0	0	0	0
Venezuela	33,654	37,586	26,716	21,101	83,180	26,413
USA (Hawaii)	—	—	—	—	96	3
<b>Total</b>	<b>421,998</b>	<b>741,794</b>	<b>322,256</b>	<b>400,519</b>	<b>652,212</b>	<b>869,087</b>

1 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1997.htm](http://www.paho.org/english/hcp/hct/dengue_1997.htm)

2 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1998.htm](http://www.paho.org/english/hcp/hct/dengue_1998.htm).

3 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1999.htm](http://www.paho.org/english/hcp/hct/dengue_1999.htm).

4 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2000.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2000.htm)

5 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2001.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2001.htm)

6 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2002.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2002.htm)

—=Data not available.

## Burden from dengue

The health and economic burden posed by dengue in the Americas has probably been underestimated because the number of deaths has been relatively low and the infection is often not diagnosed. A recent analysis showed that the impact of dengue in Latin America and the Caribbean as calculated by disability-adjusted life years (DALYs) was considerable. Meltzer et al. assessed DALYs lost to dengue in Puerto Rico for the period 1984 to 1994, using DALYs as a non-monetary measure of the economic impact<sup>(31)</sup>. They found that in Latin America and the Caribbean, the loss of DALYs per million persons from dengue fever was similar to that from malaria and from a cluster of childhood diseases (polio, measles, pertussis, diphtheria, tetanus).

Cuba provides an especially good case study because of sequential outbreaks caused by different dengue serotypes and good surveillance of clinical disease. In 1977, Cuba experienced an epidemic of DEN-1, with an estimated 4.5 million cases but no DHF or DSS. A subsequent epidemic of DEN-2 followed in 1981, causing more than 10,000 cases of DHF/DSS and 158 deaths<sup>(13)</sup>; 95% of the cases of DHF/DSS occurred in persons with secondary dengue infections. In 1997, another outbreak of DEN-2 occurred in Santiago de Cuba, causing more than 5000 cases of laboratory-confirmed DEN-2 infections, including 205 cases of DHF and 12 deaths<sup>(32,33)</sup>. In this outbreak more than 98% of those with dengue fever and DHF had secondary dengue infections. Severe dengue did not occur in anyone 18 years and younger. This

observation suggested that previous infection was the major risk factor, and also that increased risk for DHF was still present 16-20 years after the primary infection, in this case DEN-1<sup>(34)</sup>. Also notable in this epidemic was that almost all (97%) primary DEN-2 infections were mild or inapparent and were identified only by serological testing<sup>(33)</sup>.

Dengue in the United States is largely an imported infection. As suggested by the studies noted above, the infection is probably under-recognized and under-reported. In 1997 and 1998, 143 persons with laboratory-diagnosed dengue were reported. Among the 122 patients for whom a travel history was available, 61 infections were probably acquired in the Caribbean islands, 30 in Asia, 23 in Central America, 4 in South America and 3 in Africa<sup>(35)</sup>. The distribution reflects the travel patterns of US travellers and not the relative intensity of transmission in different geographical regions. One patient died; DEN-2 was documented on immunohistochemistry on autopsy tissue. The number of cases reported in 1998 was more than double the number reported in 1997. In 1999 and 2000, the number of documented cases in US travellers dropped to 41 cases<sup>(36)</sup>.

## Border areas

A study of the populations along the US-Mexico border has been instructive in trying to understand the risk factors for infection and the dynamics of its spread. In 1986, at least 9 cases of laboratory-diagnosed dengue in Texas were acquired locally. In 1995, during another period of intense transmission in Mexico (and with the

circulation of all 4 dengue serotypes in Mexico), of the 23 cases of laboratory-confirmed dengue in Texas between September and mid-November, at least 7 had been acquired in Texas. Both *Aedes aegypti* and *Aedes albopictus* were abundant in south Texas<sup>(37)</sup>.

From January to July 1999, at a time when an outbreak of more than 300 cases had been reported in Nuevo Laredo, Tamaulipas, Mexico, no cases of dengue fever were reported across the border in Laredo, Texas, USA. Each month, an estimated 2 million border crossings occur between Laredo and Nuevo Laredo. *Aedes aegypti* infests both cities. The Texas Health Department undertook a review of medical records to assess whether dengue fever was absent in Texas or whether it was unrecognized or unreported<sup>(38)</sup>. They reviewed the medical records from patients who presented themselves to five medical facilities with febrile illness (with arthralgia, myalgia, rash or headache) during a 4-week period. When they collected blood samples and tested for dengue, they found that almost 50% of those tested had serological evidence of recent dengue infection. Most, but not all, reported recent travel to Mexico within the 2 weeks prior to the onset of symptoms. Diagnoses that had been recorded for the patients at the time they were evaluated included viral syndrome and flu-like illness. After the Texas Department of Health issued an alert about dengue, 161 cases of suspected dengue were reported from mid-August through December 1999, and 18 were documented to be of dengue fever.

## Underdiagnosis and misdiagnosis

Although 4 serotypes of dengue virus exist, there are also multiple genotypes<sup>(39,40)</sup>, and these may differ in clinical manifestations and potential for enhancement with subsequent dengue infections. Furthermore, the consequences of previous infection with non-dengue flaviviruses on susceptibility to dengue and clinical expression of dengue, and vice versa, are unclear. Studies looking at these interactions are limited and are complicated by difficulties in the serological diagnosis of dengue and related flavivirus infections.

Active surveillance programmes document that dengue infections may be more common than recognized. A laboratory-based active surveillance programme for dengue in Florida in 1997-1998 detected 18 cases of dengue, all in persons who had recently travelled to dengue endemic areas<sup>(41)</sup>. Infections involved all four serotypes. In the preceding 10 years an average of 1.3 cases per year had been detected with a passive surveillance system. This is relevant because this area of Florida is inhabited by two competent vectors, *Aedes aegypti* and *Aedes albopictus*<sup>(42)</sup>.

Many cases of dengue infection are not identified because they are asymptomatic or mild or resemble many other febrile illnesses. Silent transmission of dengue viruses is more likely in populations without previous dengue, especially if caused by "low virulence" strains. For example, primary DEN-2 tends to cause mild disease, in contrast to DEN-1, which has caused highly visible epidemics in virgin

populations. Primary infections with some strains of DEN-2 and DEN-4 are largely subclinical in some settings. In Cuba, for example, 97% of the primary DEN-2 infections were clinically inapparent. In Haiti where the annual infection rate is about 30% and 85% of the residents have antibodies to two or more dengue serotypes, there have been no major outbreaks of DHF<sup>(43)</sup>. In Iquitos, Peru, DEN-1 was introduced in 1990, followed by the circulation of DEN-2 in 1995. Among the 129 students for whom serum samples were available before and after the 1995 epidemic, 60.5% were found to have secondary DEN-2 infections, caused by the American DEN-2 genotype. No cases of DHF or DSS were found, suggesting that even secondary infections with some genotypes were unlikely to cause complicated dengue<sup>(44)</sup>.

Dengue infections and even outbreaks can be missed if they are occurring in the setting of other febrile illnesses with overlapping clinical features. Several studies confirm this observation. In a serological study of diseases causing rash in Rio de Janeiro between 1994 and 1998 (set up to assess measles status in the population), investigators found that dengue was the most common diagnosis causing fever and rash<sup>(45)</sup>. Among the 71.3% patients with a confirmed diagnosis (out of 327 patients with rash diseases) 33% had dengue fever, 20.2% had rubella, 9.2% had parvovirus, 6.7% had measles and 4.2% had serological evidence of infection with HHV-6. At the time of the study, 67.6% of the cases of dengue in the area occurred in persons 15 years or older. Other studies also confirm confusion between dengue and rubella<sup>(46)</sup> and rickettsial infections<sup>(47)</sup>.

In distinguishing dengue from other febrile illnesses, it is important to know what other infections dengue can resemble. Several studies and observations illustrate how dengue fever can be missed or misdiagnosed. In Barbados, the peak of leptospirosis coincides with the increase in rainfall. This island also experienced dengue epidemics in 1995 and 1997. In 1995 and 1997, among the patients investigated for leptospirosis, more were found to have dengue than leptospirosis<sup>(48)</sup>.

On the other hand, in a large urban outbreak of leptospirosis in Salvador, Brazil, in 1994, 43% of the patients with confirmed or probable leptospirosis were initially diagnosed as having dengue fever<sup>(49)</sup>. In late 1994 hundreds of people in a rural area north of Lake Managua, Nicaragua developed acute illness with fever, headaches and muscle aches, and fatalities resulted from respiratory distress and pulmonary haemorrhage. Again, the initial focus was on dengue haemorrhagic fever, but the dengue studies were negative. Additional studies revealed leptospirosis as the cause. Heavy rains and flooding had preceded the outbreak<sup>(50)</sup>.

Another outbreak of haemorrhagic fever, which occurred in Guaranito, Portuguesa State, Venezuela, in 1989, was initially thought to be dengue haemorrhagic fever – but studies for dengue were negative. Subsequent studies identified a previously unrecognized virus, an arenavirus designated as Guaranito virus, as the cause of the disease, later called Venezuelan haemorrhagic fever<sup>(51)</sup>. The source of the virus was the cotton rat. Development of new agricultural lands and migration of large



numbers of workers into the area had increased the likelihood of human-rodent contact.

When military troops were deployed in Haiti starting in 1994, routine medical surveillance was carried out at 22 military clinics<sup>(52)</sup>. Among the 406 combat support hospital admissions during the first 6 months of deployment, 25% were due to febrile illnesses. More detailed diagnostic studies showed that dengue fever accounted for at least 30% of febrile illnesses leading to hospitalization. DEN-1, DEN-2 and DEN-4 were isolated from the troops. The clinicians observed that they were unable to diagnose dengue based on clinical grounds alone<sup>(53)</sup>.

## Clinical highlights

The clinical presentations of dengue in the Americas are generally similar to the cases in south-east Asia. However, the age distribution of DHF cases in the Americas differed somewhat from Asia. In Puerto Rico in 1990-1991 the mean age of patients who developed DHF was 38, whereas in south-east Asia primarily young children become ill with DHF<sup>(54)</sup>. In the 1994 epidemic of dengue fever and dengue haemorrhagic fever in Ceara State, Brazil, the mean age of DHF cases was 42<sup>(55)</sup>. In the 1990-91 study of 56 confirmed cases of DHF in Rio de Janeiro, Brazil, the modal age range was 31-45<sup>(56)</sup>. Likewise, in the 1997 outbreak in Cuba, DHF primarily affected young adults<sup>(33)</sup>. The overall case-fatality rate of DHF in the Americas from 1997-2002 ranges from 0.9% to 1.6%<sup>(24,57,58,59,60,61)</sup>.

## New approaches to surveillance

In Puerto Rico dengue is a well-known and common infection, and surveillance systems are in place. In contrast, leptospirosis is rarely reported, though a seroprevalence study found antibodies in 14% of the population. After Hurricane Hortense hit the island in 1996, resulting in heavy rains and flooding, leptospirosis was diagnosed in patients initially suspected to have dengue fever. This led investigators to do a study using an island-wide dengue laboratory-based surveillance system. Serum samples from patients with suspected dengue that tested negative for dengue were subsequently tested for the evidence of leptospirosis. They found that before the hurricane, 6% of the dengue-negative patients have had leptospirosis. This increased to 24% (17/70) during the post hurricane period<sup>(62)</sup>.

In French Guiana investigators tested a laboratory surveillance system to try to find a way to identify a dengue epidemic early in its course<sup>(63)</sup>. Because the area is endemic for malaria and malaria smears are typically included as part of the work up of a febrile patient, they monitored the number of studies for malaria that were negative for malaria as a way to capture non-specific febrile illnesses. In that setting, the number of negative malaria studies was found to be a good predictor of dengue fever in some towns. This underscores the need to have baseline information about diseases present in an area and to base surveillance systems on local or regional characteristics relevant to the population.

## Conclusions and recommendations

Dengue is spreading geographically in the Americas, reaching larger populations and causing a more severe disease in areas that have concurrent or sequential circulation of multiple serotypes. Sustained control of the vector is difficult to achieve. Programmes to date have had limited sustained success, except perhaps in focal areas.

Better rapid diagnostic tests are needed. Also needed are more complete epidemiological studies by geographical area which would provide a profile of common infections in each area that can resemble dengue fever. Experience gained from outbreak investigations and surveillance studies, as described above, has shown that diseases as diverse as leptospirosis,

Venezuelan haemorrhagic fever, influenza, rubella, measles, and rickettsial infections have been misdiagnosed as dengue fever, or vice versa. Mechanisms of transmission, approaches to treatment, and control measures vary substantially among these infections, making accurate and timely diagnoses essential to provide informed interventions.

Perhaps because dengue fever is often not diagnosed or reported, the impact of the disease has been underestimated. Given the current circumstances in the Americas (population location, density, mobility, living conditions and current status of vector distribution and density) and what is known about the complexity of dengue viruses and the immune response they induce, it is likely that dengue fever will grow in importance in the Americas in the coming decades.

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# Dengue in the Americas

by

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## Abstract

Dengue has been present in the Americas for centuries, but the current situation is dynamic, and is worsening. The main vector, *Aedes aegypti*, infests most countries. Vector control efforts have been largely inadequate. Population growth, especially in urban, low latitude areas, has resulted in a greater risk for contact with a competent vector. An increasing number of cities have reached a size that can support ongoing circulation of dengue viruses. The mobility and increasing connectedness of human populations through rapid international air travel permit regular introductions of the dengue virus via viremic travellers. More geographic areas have concurrent or sequential circulation of more than one serotype, increasing the risk for severe and complicated dengue.

Serological studies document that dengue infections frequently go unrecognized. Other infections, some treatable with specific antibiotics, are often misdiagnosed as dengue fever, and dengue fever is often presumptively diagnosed when other infections are the cause of illness. Rapid, reliable and inexpensive diagnostic tests are unavailable in most areas. Analyses of illness and death related to dengue show that the burden from dengue in tropical and subtropical America is substantial and is often underestimated. The health and economic impact from dengue is similar to that from other infections that have generated much more attention. Study of border areas that share similar geoclimatic conditions (for example, Mexico and the United States) can help identify risk factors and possible protective measures. Well-studied island epidemics (e.g. Cuba) have been valuable in defining the role of sequential infections, duration of relevant immunological memory, and potential impact of genotype in addition to serotype on the clinical course of the disease.

**Keywords:** Dengue, Dengue haemorrhagic fever, epidemiology, vector control, Americas.

## Introduction

Dengue has long been present in the Americas. Historical records suggest that dengue outbreaks may have occurred in the French West Indies and Panama in the

1600s, in Peru, Mexico, Colombia, the United States (Philadelphia) and the Caribbean in the 1700s, and, in addition, in Brazil, Venezuela, and Argentina in the 1800s and the first half of the 1900s<sup>(1)</sup>. Since the 1960s, dengue virus has spread to

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previously unaffected areas in the Americas and threatens a larger population than ever before in the Americas. Circulation of more than one serotype has been documented in more and more regions, increasing the risk of serious and complicated infections, i.e. dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF)<sup>(2,3)</sup>. Trends in the region, including increasing urbanization, increasing travel and migration of humans, and expansion of the areas infested (and reinfested) by competent vectors, suggest that dengue will continue to be an important cause of morbidity and mortality in the region<sup>(4,5,6,7)</sup>. This paper describes recent dengue activity in the Americas and discusses some of the features of its epidemiology and spread in the Western Hemisphere. A description of the 2001-2002 outbreak of dengue in Hawaii, transmitted by *Aedes albopictus*, is not included in this paper. Preliminary reports describing the outbreak in Hawaii are available from the publications of the Centers for Disease Control, Atlanta, and its website ([www.cdc.gov/travel/other/dengue-hawaii.oct2001.htm](http://www.cdc.gov/travel/other/dengue-hawaii.oct2001.htm)).

## History of dengue in the Americas

More than 2.5 billion people, or about 40% of the global population, live in dengue-endemic regions, which encompass tropical and sub-tropical regions worldwide. An estimated 50 to 100 million cases of DF occur each year, including several hundred thousand cases of dengue haemorrhagic fever (DHF)<sup>(4,5)</sup>. Most go unreported. Reports to WHO in 1998 recorded 1.2 million cases of dengue and DHF and 15,000 deaths. Although, globally, the greatest burden from

dengue continues to occur in Asia, the situation in the Americas is dynamic, and is worsening. In 1998, the Americas reported >700,000 cases of DF and >12,000 cases of DHF. This year (2002) Brazil has experienced the worst epidemic in its history (see below). The number of dengue cases in the Americas should be considered in the context of the population size, which is small in comparison to Asia. The population of the Americas (not all of which is located in areas at risk for dengue infection) was estimated at 823 million in 1999, representing less than 14% of the world's population. The population is distributed with about one-third residing in the US, one-third in Mexico and Brazil, and the remainder in the other 45 countries and territories<sup>(8)</sup>.

*Aedes aegypti*, the main vector for dengue in the Americas (note exception in Hawaii, 2001-2002) is believed to have been introduced into the Western Hemisphere on slave ships from West Africa in the fifteenth through the seventeenth centuries. The mosquito may also have reached the New World on European ships from Spain and Portugal. *Aedes aegypti* was well adapted to flourish in water storage containers on ships<sup>(9)</sup>. *Aedes aegypti* became established widely in tropical and temperate areas of the Americas.

An intensive campaign organized by the Pan American Health Organization (PAHO) starting in 1947 to control yellow fever transmission led to eradication of *Ae. aegypti* from all countries in the Americas except the U.S., Suriname, Venezuela and several Caribbean Islands by the late 1960s<sup>(1,4)</sup>. Records show no evidence of epidemic dengue in the Americas from 1946

through 1963, presumably reflecting in part the benefits from the eradication programme. In areas where *Aedes aegypti* was eliminated, transmission of dengue virus was interrupted. Support for programmes waned and vector control activities declined allowing the mosquito to reinfest areas where it had been eliminated and to spread to areas where it had never previously been recorded. *Aedes aegypti* was repeatedly introduced from areas where the vector was not controlled. Dengue re-emerged in the 1960s and 1970s, initially affecting Jamaica, then Puerto Rico, other Caribbean islands, and Venezuela<sup>(10,11,12)</sup>. Subsequently, dengue

was reported from at least 43 countries in the region<sup>(1,4,5,6,7,10)</sup>. Outbreaks of classic dengue appeared, but these were followed by the appearance of DHF, initially in a massive epidemic in Cuba in 1981<sup>(10)</sup> and since then in other parts of the Caribbean and Central and South America<sup>(13)</sup>. The second major DHF epidemic occurred in 1989-1990 in Venezuela, when 3,108 cases of DHF were identified<sup>(14)</sup>. Table 1 provides a chronological listing of the country locations where different dengue serotypes have been documented, starting in 1953.

**Table 1.** The introduction of dengue serotypes (DEN-1, 2, 3, 4) in the Americas: predominant serotypes, arrival of new strains, countries in which they were identified and associated

Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1953		Trinidad			First-ever isolation of DEN-2 in the Americas; no outbreak was reported <sup>(iii)</sup> .
1963-64			Jamaica Puerto Rico Lesser Antilles Venezuela		A Caribbean pandemic occurred <sup>(iv)</sup> . > 27,000 cases occurred in Puerto Rico <sup>(v)</sup> .
1968-69		Caribbean Venezuela	Caribbean Venezuela		DEN-2 and DEN-3 were found in Jamaica <sup>(vi)</sup> .
1971-72		Colombia			<i>Aedes aegypti</i> reinfested the country, followed by a dengue epidemic <sup>(vii)</sup> .
1975-77			Colombia		A second dengue epidemic occurred in Colombia <sup>(vii)</sup> .
1977	Jamaica Cuba				<b>DEN-1 was introduced, and spread in Caribbean, South America, Central America, and led to a pandemic<sup>(viii)</sup>.</b>
1978	Venezuela Colombia Guyana Suriname French Guiana Honduras El Salvador Guatemala Belize Mexico				From 1977-80, 702,000 cases were reported to PAHO from the Americas, but estimates from Colombia, Cuba, and Venezuela suggested >5 million cases <sup>(ix)</sup> . Epidemic in Central America affected Honduras <sup>(x)</sup> , followed by El Salvador, Guatemala, Belize, and spread to Mexico.

Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1980	Texas, USA				Most cases occurred near the Texas-Mexico border <sup>(xi)</sup> .
1981		Cuba		Caribbean S. America C. America Mexico	New DEN-2 strain was introduced in Cuba; <b>1<sup>st</sup> major DHF epidemic</b> , total of 344,203 cases, 10,312 severe, 158 fatal <sup>(xii)</sup> . DEN-4 was introduced <sup>(xiii)</sup> , affecting the Caribbean, northern South America, Central America, and Mexico.  8350 cases <sup>(i)</sup>
1982	Colombia Brazil			Colombia Brazil El Salvador Puerto Rico	6776 cases <sup>(i)</sup> > 12,000 cases <sup>(i)</sup> 5166 cases <sup>(i)</sup> 9536 <sup>(1)</sup>
1983	Colombia  Mexico	Jamaica Trinidad Mexico		Colombia El Salvador Jamaica Trinidad	4977 cases <sup>(xiv)</sup> 3814 cases <sup>(i)</sup> DEN-2 predominated <sup>(xiv)</sup> DEN-4 predominated <sup>(xiv)</sup> 19,028 cases <sup>(i)</sup>
1984					DEN-1,2,4 continued to circulate, causing 43,435 cases in the region <sup>(xv)</sup> .
1985	Nicaragua Aruba Honduras El Salvador Venezuela Colombia Puerto Rico	Nicaragua  Venezuela Colombia Puerto Rico		Venezuela	17,483 cases <sup>(xv,xvi)</sup> 24,000 cases <sup>(i)</sup>     7797 cases <sup>(xv)</sup>
1986	Brazil  Puerto Rico	  Puerto Rico		  Puerto Rico	47,370 cases; major outbreak occurred in Rio de Janeiro <sup>(xvii,xviii)</sup> .  10,659 cases, predominantly DEN-4 <sup>(xix)</sup> , followed by continued outbreaks ever since.
1987	<b>Bolivia</b>				1994 cases and 4847 cases the following year <sup>(i)</sup> .
1988	Paraguay  Ecuador				405 cases followed by 41,800 cases the following year <sup>(i)</sup> . Epidemic occurred in Guayaquil, following <i>Aedes aegypti</i> reinfestation (detected in 1985) <sup>(xx)</sup> .
1989-90	Venezuela	Venezuela		Venezuela	<b>2<sup>nd</sup> DHF epidemic in the Americas</b> – 3108 cases of DHF, 73 deaths, with annual epidemics ever since; DEN-2 was predominant <sup>(xxi)</sup> .
1990	Peru  Brazil	  Brazil		Peru	<b>7858 cases, followed by continued transmission<sup>(i,xxii)</sup>.</b> <i>Epidemic occurred in the state Rio de Janeiro<sup>(xiii)</sup>.</i>



Year	DEN-1	DEN-2	DEN-3	DEN-4	Comments and References <sup>(i,ii)</sup>
1991-1992		French Guiana			DEN-2 predominated although DEN-1 was also present <sup>(xiv)</sup> .
1993	Costa Rica	<b>Panama</b>			1 <sup>st</sup> outbreak since 1942 <sup>(xv)</sup> 4612 cases <sup>(xvi)</sup> .
1994		Brazil Puerto Rico			54,453 cases, reaching 530,578 cases by 1998 <sup>(i,xvii)</sup> . 22,000 cases <sup>(i)</sup> .
	Panama Costa Rica		Nicaragua Panama Costa Rica		Reappearance of DEN-3; total 20,469 cases; 1247 cases DHF; incidence 4.8/1000 <sup>(xv)</sup> . DEN-3 was of the Sri Lanka/India genotype, DEN-1 was predominant <sup>(xv)</sup> . 13,929 cases <sup>(i)</sup>
1995			Mexico Guatemala		DEN-3 identified in Mexico <sup>(xviii)</sup> . Total of 316,411 cases occurred in the Americas <sup>(i)</sup> . DEN-3 predominated for next 3 years <sup>(xix)</sup> .
1996-1997	Brazil	Brazil			Epidemic in Belem, Para State <sup>(xx)</sup> .
1997		Cuba Argentina			DEN-2 re-emerged in Cuba; 2946 laboratory-confirmed cases; 205 DHF/DSS <sup>(xxi)</sup> . 1 <sup>st</sup> cases since 1916 were identified <sup>(xxii)</sup> .
1998		Nicaragua	Nicaragua		DEN-3 predominated <sup>(xxiii)</sup> .
2000	Costa Rica	El Salvador Guatemala Honduras Nicaragua	Nicaragua Costa Rica Brazil		<b>All serotypes are present in the 1990s<sup>(xxiv)</sup>.</b> <b>16,355 cases</b> <b>5963 cases</b> <b>8715 cases</b> <b>5233 cases</b> <b>2113 cases</b> DEN-3 appeared in Brazil in 2000 <sup>(xxv)</sup> .
2002	Colombia El Salvador	Colombia El Salvador	Brazil Colombia El Salvador	Colombia El Salvador	Huge epidemic; 711,919 cases reported, 2229 DHF, 130 deaths as of September 15 <sup>(xxvi,xxvii)</sup> . DEN-1, 2, 3, 4 are reported to be circulating in Colombia and El Salvador <sup>(xxviii)</sup> . Epidemics have also been reported from Honduras, Nicaragua, Venezuela <sup>(xxviii)</sup> .

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Programmes to control vectors face new challenges. Resistance of vectors to insecticides is increasing, limiting the available options. Resistance of *Aedes aegypti* to pyrethroids is now widespread<sup>(15)</sup>. Other factors have also contributed to increased vector abundance, among them expansion of water storage locations and a plethora of vector breeding sites in used tyres, flower pots, and discarded plastic cups and other non-biodegradable containers. Inadequate piped water supplies lead people to store water in and around homes.

## Human population

The human population has also grown but more relevant is the location of the population expansion. Most of the population growth has occurred in urban areas. Globally, more people now live in urban areas than ever before. In South America, 78% of the population lived in urban areas in 1995; this is projected to increase to 88% by 2025. (The comparable figures for the world are 45% in 1995 and 60% by 2025.) Much of the growth of cities is unplanned and unregulated, and huge slums surround some cities. Many of these areas are characterized by poor housing,

crowded living conditions, inadequate waste disposal and lack of clean water. This contemporary landscape provides an ideal environment for breeding of *Aedes aegypti*, a mosquito that is well adapted to the urban habitat. Poor housing and absence of screens increase the potential contact between mosquitoes and humans. More urban centres have reached a population size that it takes to sustain the ongoing circulation of a dengue virus in a population, which increases the risk of severe forms of dengue.

Recent studies also point to another reason to be concerned about population size. Over the past two centuries the number of dengue lineages has been increasing roughly in parallel with the size of the human population<sup>(16)</sup>. Scientists have suggested that a larger human population in which the virus replicates allows increased opportunities for viral evolution and enhances the potential for the appearance of more virulent strains. Intra-serotype recombination events may also play an important role in genetic diversity of the dengue virus<sup>(17)</sup>. More opportunities for such genetic exchange exist in populations with hyperendemic dengue.

This large and growing urban population in low latitude areas is increasingly linked by air travel to other areas of the world. Global travel continues to increase. In 1996, 2.5 billion persons passed through airports and more than 500 million persons crossed international borders on commercial airplane flights. Air traffic volume has increased about 7% per year for the past 20 years. As of the mid-1990s, about 5000 airports had scheduled worldwide service. This massive movement of the human population links all major urban areas of the world. Speed of travel means that a person bitten by an infective mosquito (carrying dengue) in Thailand can reach home in Brazil before the symptoms of the infection begin to show. Viremia, which may persist for as long as 7-8 days, typically begins 2 days before the onset of the symptoms, during which the viremic human may continue his usual activities and may encounter competent vectors at work, home and elsewhere. Levels of virus in blood may be high. In a study of plasma viral RNA levels in children in Thailand, levels ranged as high as  $10^9$  RNA copies/ml, peaked an average of 2 days before defervescence, and dropped rapidly during the last several days of fever<sup>(18)</sup>. Table 2a contains the tourist arrivals to a number of the countries in the Americas that have had the highest volumes of visits, as well as the recent incidence of dengue in the local population as reported to the Pan American Health Organization (PAHO). Costa Rica reported the population at risk, and the incidence was calculated based on that figure. Bolivia reported the population at risk for 2001 also. The other countries in the table reported projected populations. Table 2b lists international arrivals in each

sub-region. These figures indicate that dengue-endemic countries in the Americas receive over 50 million international arrivals each year.

**Table 2a:** International arrivals in some dengue-endemic countries in the Americas

Country	Inter-national arrivals in 2000 <sup>a</sup>	Incidence of dengue in local population (per 100,000) <sup>b</sup>	
		2000	2001
Argentina	2,949,000	4.59	0.03
Bahamas	1,577,000	0	
Brazil	5,313,000	136.07	239.38
Costa Rica	1,088,000	434.63 <sup>c</sup>	818.163 <sup>c</sup>
Cuba (air arrivals only)	1,741,000	1.23	101.58
Dominican Republic (air arrivals only)	2,972,000	40.75	42.28
Jamaica (air arrivals only)	1,323,000	0.97	1.50
Mexico	20,641,000	21.96	6.19
Peru	1,027,000	21.38	89.41
Puerto Rico (air arrivals only)	3,341,000	62.88	132.41

<sup>a</sup>From World Tourism Organization News Release, [www.world-tourism.org/newsroom/releases/more\\_releases/january2002/numbers\\_2001.htm](http://www.world-tourism.org/newsroom/releases/more_releases/january2002/numbers_2001.htm)

<sup>b</sup>PAHO. [www.paho.org/English/HCP/HCT/VBD/dengue-cases-2000.htm](http://www.paho.org/English/HCP/HCT/VBD/dengue-cases-2000.htm) and [www.paho.org/English/HCP/HCT/VBD/dengue-cases-2001.htm](http://www.paho.org/English/HCP/HCT/VBD/dengue-cases-2001.htm).

<sup>c</sup>Based on population at risk.

**Table 2b:** International arrivals in sub-regions in the Americas in 2000

Sub-Region	Arrivals in 2000 (millions) <sup>a</sup>
North America	91.2 (Canada 19.7; USA 50.9)
Caribbean	17.3
Central America	4.4
South America	15.5 (Chile 1.7; Uruguay 2.0)
Total for Americas	128.3

<sup>a</sup>From World Tourism Organization News Release, [www.world-tourism.org/newsroom/releases/more\\_releases/january2002/numbers\\_2001.htm](http://www.world-tourism.org/newsroom/releases/more_releases/january2002/numbers_2001.htm)

## Vector populations

Shipping and trade have allowed the introduction of *Aedes albopictus*, also a competent vector for dengue, into previously uninfested countries. In 1985, *Aedes albopictus* was introduced into North America, probably via used tyres shipped from north Asia. It was first found in Texas and within 12 years had spread to 678 counties in 25 states<sup>(19,20)</sup>. Its dispersal followed inter-state highways, and presumably was carried with human traffic and trade. As of the late 1990s, *Aedes albopictus* had been introduced into Brazil, the Dominican Republic, Guatemala, Mexico, Cuba, Bolivia, El Salvador and Colombia, and was continuing to spread<sup>(21)</sup>. Modern ships, especially those with container vessels, are effective in dispersing the immature stages of mosquitoes. Most successful mosquito invasions have resulted from ship transport. Desiccation-resistant *Aedes* eggs are effectively transported in tyres. The reinfestation of *Aedes aegypti* and the establishment of *Aedes albopictus* in the Americas provide the vehicles for major

epidemics of dengue fever and dengue haemorrhagic fever in this region.

## Recent dengue outbreaks in specific countries

All four serotypes have circulated widely and continue to cause dengue outbreaks in the Americas<sup>(22)</sup>. All four serotypes have been associated with epidemics in the 1990s in Costa Rica, El Salvador, Guatemala, Honduras and Nicaragua. As of early 1999 all four serotypes had also been documented in Mexico and Puerto Rico<sup>(23,24)</sup>. As of September 15, 2002, numerous countries have reported dengue and DHF to PAHO, including Brazil, Colombia, Costa Rica, Cuba, El Salvador, Honduras, Peru, Venezuela, Barbados, and Trinidad & Tobago<sup>(25)</sup> (See Table 3).

In Brazil, more than 500,000 dengue cases were reported in 1998 and *Aedes aegypti* infested all important urban centres of the country. DEN-1 has been present since 1986 and DEN-2 since 1990<sup>(26,27)</sup>, setting the stage for widespread DHF/DSS if new serotypes of dengue are introduced. As of September 2002, more than 711,919 cases of dengue (predominantly DEN-3, although DEN-1 and DEN-2 are also present) as well as 2,229 cases of DHF and 130 deaths had been reported in the country<sup>(25)</sup>. The dengue epidemic affected Rio de Janeiro state most severely<sup>(28)</sup>.

*Aedes aegypti* reinfested Argentina as far south as Buenos Aires after the vector had been eradicated in 1963. In 1996 and 1997, high levels of infestation were noted in Buenos Aires province and the Federal District. In 1997, viremic travellers brought dengue virus to Argentina and local transmission occurred in

Salta province in northern Argentina. This was the southern most extension of dengue noted to that point<sup>(29)</sup>.

Similarly, Easter Island, Chile and Hawaii (USA) recently suffered outbreaks of dengue. Both outbreaks were caused by DEN-1, and probably had been spread by travellers from Tahiti, the American Samoa or Western Samoa. More than 100 cases of dengue have been reported from Hawaii since 2001<sup>(30)</sup> and more than 130 cases have been reported from Easter Island in 2002<sup>(25)</sup>. Although small in comparison to the outbreaks elsewhere in the Americas, these

mark new territories of dengue invasion in 2001-2002.

Table 3 lists dengue cases by country from 1997 to 2002. Note that Brazil, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Peru, Puerto Rico and Venezuela have had major dengue outbreaks or epidemics during this period. Over 320,000 cases of dengue fever were reported annually from the region since 1997. In the first quarter of 2002, there were already more than 400,000 cases reported.

**Table 3: Countries in the Americas and reported dengue cases (1997-2002)**

Country or Region	1997 <sup>1</sup>	1998 <sup>2</sup>	1999 <sup>3</sup>	2000 <sup>4</sup>	2001 <sup>5</sup>	2002 (as of September 15) <sup>6</sup>
Anguilla	0	0	12	3	25	10
Antigua& Barbuda	7	4	2	8	20	14
Argentina		822	3	1,700	11	234
Aruba			130	76	0	28
Bahamas	0	336	0	0	0	0
Barbados	199	852	696	744	1,043	488
Belize	141	8	3	4	3	19
Bermuda	0	0	3	0	0	0
Bolivia	539	49	43	73	176	661
Bonaire	—	—	—	—	—	—
Brazil	254,109	535,388	204,201	231,471	413,067	711,919
British Virgin Islands	0	1	0	3	23	7
Cayman Islands	0	2	1	0	0	1
Chile (Easter Island)	—	—	—	—	—	636
Colombia	24,290	63,182	20,336	22,775	55,437	34,559
Costa Rica	14,267	2,628	6,040	4,907	9,237	5,509
Cuba	3,012	—	0	138	11,432	3,011
Curacao	—	7	5	10	0	—
Dominica	0	1	1	15	5	0
Dominican Republic	608	3,049	1,088	3,462	3,592	736
Ecuador	3,871	4,606	2,901	22,937	10,919	5,833

Country or Region	1997 <sup>1</sup>	1998 <sup>2</sup>	1999 <sup>3</sup>	2000 <sup>4</sup>	2001 <sup>5</sup>	2002 (as of September 15) <sup>6</sup>
El Salvador	423	1688	626	3248	1093	17,123
French Guiana	851	534	88	186	2830	
Grenada	22	4	85	27	12	221
Guadeloupe	0		41	60	0	
Guatemala	5,385	4,655	3,617	9,006	4,516	3,986
Guyana	0	42	0	19	60	9,180
Haiti	—	—	—	—	—	—
Honduras	11,873	22,218	17,835	13,642	9,077	27,113
Jamaica	16	1,551	24	25	39	27
Martinique	235	44	268	171	4,471	
Mexico	53,541	23,639	14,875	21,715	6210	3005
<b>Montserrat</b>	—	0	0	9	1	4
Netherland Antilles	—	—	—	—	—	—
Nicaragua	3,126	13,592	11,150	7,317	2,104	4,258
Panama	2,628	2,717	2,783	317	1,545	405
Paraguay			1,164	24,282	38	1,795
Peru	1,357	988	554	5,486	23,329	7,196
Puerto Rico	6,955	17,241	4,993	2,433	5,233	1,662
St. Kitts/Nevis	0		1	5	89	18
St. Lucia	12	1	3	0	292	44
St. Martin	—	—	—	—	—	—
St. Vincent & Grenadines	3	88	8	5	3	124
Suriname	90	1,151	695	1,073	760	220
Trinidad & Tobago	784	3,120	1,265	2,066	2,244	2,625
Turks & Caicos Islands	0	—	0	0	0	0
Venezuela	33,654	37,586	26,716	21,101	83,180	26,413
USA (Hawaii)	—	—	—	—	96	3
<b>Total</b>	<b>421,998</b>	<b>741,794</b>	<b>322,256</b>	<b>400,519</b>	<b>652,212</b>	<b>869,087</b>

1 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1997.htm](http://www.paho.org/english/hcp/hct/dengue_1997.htm)

2 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1998.htm](http://www.paho.org/english/hcp/hct/dengue_1998.htm).

3 PAHO. [www.paho.org/english/hcp/hct/dengue\\_1999.htm](http://www.paho.org/english/hcp/hct/dengue_1999.htm).

4 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2000.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2000.htm)

5 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2001.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2001.htm)

6 PAHO. [www.paho.org/english/hcp/hct/vbd/dengue-cases-2002.htm](http://www.paho.org/english/hcp/hct/vbd/dengue-cases-2002.htm)

—=Data not available.

## Burden from dengue

The health and economic burden posed by dengue in the Americas has probably been underestimated because the number of deaths has been relatively low and the infection is often not diagnosed. A recent analysis showed that the impact of dengue in Latin America and the Caribbean as calculated by disability-adjusted life years (DALYs) was considerable. Meltzer et al. assessed DALYs lost to dengue in Puerto Rico for the period 1984 to 1994, using DALYs as a non-monetary measure of the economic impact<sup>(31)</sup>. They found that in Latin America and the Caribbean, the loss of DALYs per million persons from dengue fever was similar to that from malaria and from a cluster of childhood diseases (polio, measles, pertussis, diphtheria, tetanus).

Cuba provides an especially good case study because of sequential outbreaks caused by different dengue serotypes and good surveillance of clinical disease. In 1977, Cuba experienced an epidemic of DEN-1, with an estimated 4.5 million cases but no DHF or DSS. A subsequent epidemic of DEN-2 followed in 1981, causing more than 10,000 cases of DHF/DSS and 158 deaths<sup>(13)</sup>; 95% of the cases of DHF/DSS occurred in persons with secondary dengue infections. In 1997, another outbreak of DEN-2 occurred in Santiago de Cuba, causing more than 5000 cases of laboratory-confirmed DEN-2 infections, including 205 cases of DHF and 12 deaths<sup>(32,33)</sup>. In this outbreak more than 98% of those with dengue fever and DHF had secondary dengue infections. Severe dengue did not occur in anyone 18 years and younger. This

observation suggested that previous infection was the major risk factor, and also that increased risk for DHF was still present 16-20 years after the primary infection, in this case DEN-1<sup>(34)</sup>. Also notable in this epidemic was that almost all (97%) primary DEN-2 infections were mild or inapparent and were identified only by serological testing<sup>(33)</sup>.

Dengue in the United States is largely an imported infection. As suggested by the studies noted above, the infection is probably under-recognized and under-reported. In 1997 and 1998, 143 persons with laboratory-diagnosed dengue were reported. Among the 122 patients for whom a travel history was available, 61 infections were probably acquired in the Caribbean islands, 30 in Asia, 23 in Central America, 4 in South America and 3 in Africa<sup>(35)</sup>. The distribution reflects the travel patterns of US travellers and not the relative intensity of transmission in different geographical regions. One patient died; DEN-2 was documented on immunohistochemistry on autopsy tissue. The number of cases reported in 1998 was more than double the number reported in 1997. In 1999 and 2000, the number of documented cases in US travellers dropped to 41 cases<sup>(36)</sup>.

## Border areas

A study of the populations along the US-Mexico border has been instructive in trying to understand the risk factors for infection and the dynamics of its spread. In 1986, at least 9 cases of laboratory-diagnosed dengue in Texas were acquired locally. In 1995, during another period of intense transmission in Mexico (and with the



circulation of all 4 dengue serotypes in Mexico), of the 23 cases of laboratory-confirmed dengue in Texas between September and mid-November, at least 7 had been acquired in Texas. Both *Aedes aegypti* and *Aedes albopictus* were abundant in south Texas<sup>(37)</sup>.

From January to July 1999, at a time when an outbreak of more than 300 cases had been reported in Nuevo Laredo, Tamaulipas, Mexico, no cases of dengue fever were reported across the border in Laredo, Texas, USA. Each month, an estimated 2 million border crossings occur between Laredo and Nuevo Laredo. *Aedes aegypti* infests both cities. The Texas Health Department undertook a review of medical records to assess whether dengue fever was absent in Texas or whether it was unrecognized or unreported<sup>(38)</sup>. They reviewed the medical records from patients who presented themselves to five medical facilities with febrile illness (with arthralgia, myalgia, rash or headache) during a 4-week period. When they collected blood samples and tested for dengue, they found that almost 50% of those tested had serological evidence of recent dengue infection. Most, but not all, reported recent travel to Mexico within the 2 weeks prior to the onset of symptoms. Diagnoses that had been recorded for the patients at the time they were evaluated included viral syndrome and flu-like illness. After the Texas Department of Health issued an alert about dengue, 161 cases of suspected dengue were reported from mid-August through December 1999, and 18 were documented to be of dengue fever.

## Underdiagnosis and misdiagnosis

Although 4 serotypes of dengue virus exist, there are also multiple genotypes<sup>(39,40)</sup>, and these may differ in clinical manifestations and potential for enhancement with subsequent dengue infections. Furthermore, the consequences of previous infection with non-dengue flaviviruses on susceptibility to dengue and clinical expression of dengue, and vice versa, are unclear. Studies looking at these interactions are limited and are complicated by difficulties in the serological diagnosis of dengue and related flavivirus infections.

Active surveillance programmes document that dengue infections may be more common than recognized. A laboratory-based active surveillance programme for dengue in Florida in 1997-1998 detected 18 cases of dengue, all in persons who had recently travelled to dengue endemic areas<sup>(41)</sup>. Infections involved all four serotypes. In the preceding 10 years an average of 1.3 cases per year had been detected with a passive surveillance system. This is relevant because this area of Florida is inhabited by two competent vectors, *Aedes aegypti* and *Aedes albopictus*<sup>(42)</sup>.

Many cases of dengue infection are not identified because they are asymptomatic or mild or resemble many other febrile illnesses. Silent transmission of dengue viruses is more likely in populations without previous dengue, especially if caused by "low virulence" strains. For example, primary DEN-2 tends to cause mild disease, in contrast to DEN-1, which has caused highly visible epidemics in virgin

populations. Primary infections with some strains of DEN-2 and DEN-4 are largely subclinical in some settings. In Cuba, for example, 97% of the primary DEN-2 infections were clinically inapparent. In Haiti where the annual infection rate is about 30% and 85% of the residents have antibodies to two or more dengue serotypes, there have been no major outbreaks of DHF<sup>(43)</sup>. In Iquitos, Peru, DEN-1 was introduced in 1990, followed by the circulation of DEN-2 in 1995. Among the 129 students for whom serum samples were available before and after the 1995 epidemic, 60.5% were found to have secondary DEN-2 infections, caused by the American DEN-2 genotype. No cases of DHF or DSS were found, suggesting that even secondary infections with some genotypes were unlikely to cause complicated dengue<sup>(44)</sup>.

Dengue infections and even outbreaks can be missed if they are occurring in the setting of other febrile illnesses with overlapping clinical features. Several studies confirm this observation. In a serological study of diseases causing rash in Rio de Janeiro between 1994 and 1998 (set up to assess measles status in the population), investigators found that dengue was the most common diagnosis causing fever and rash<sup>(45)</sup>. Among the 71.3% patients with a confirmed diagnosis (out of 327 patients with rash diseases) 33% had dengue fever, 20.2% had rubella, 9.2% had parvovirus, 6.7% had measles and 4.2% had serological evidence of infection with HHV-6. At the time of the study, 67.6% of the cases of dengue in the area occurred in persons 15 years or older. Other studies also confirm confusion between dengue and rubella<sup>(46)</sup> and rickettsial infections<sup>(47)</sup>.

In distinguishing dengue from other febrile illnesses, it is important to know what other infections dengue can resemble. Several studies and observations illustrate how dengue fever can be missed or misdiagnosed. In Barbados, the peak of leptospirosis coincides with the increase in rainfall. This island also experienced dengue epidemics in 1995 and 1997. In 1995 and 1997, among the patients investigated for leptospirosis, more were found to have dengue than leptospirosis<sup>(48)</sup>.

On the other hand, in a large urban outbreak of leptospirosis in Salvador, Brazil, in 1994, 43% of the patients with confirmed or probable leptospirosis were initially diagnosed as having dengue fever<sup>(49)</sup>. In late 1994 hundreds of people in a rural area north of Lake Managua, Nicaragua developed acute illness with fever, headaches and muscle aches, and fatalities resulted from respiratory distress and pulmonary haemorrhage. Again, the initial focus was on dengue haemorrhagic fever, but the dengue studies were negative. Additional studies revealed leptospirosis as the cause. Heavy rains and flooding had preceded the outbreak<sup>(50)</sup>.

Another outbreak of haemorrhagic fever, which occurred in Guaranito, Portuguesa State, Venezuela, in 1989, was initially thought to be dengue haemorrhagic fever – but studies for dengue were negative. Subsequent studies identified a previously unrecognized virus, an arenavirus designated as Guaranito virus, as the cause of the disease, later called Venezuelan haemorrhagic fever<sup>(51)</sup>. The source of the virus was the cotton rat. Development of new agricultural lands and migration of large

numbers of workers into the area had increased the likelihood of human-rodent contact.

When military troops were deployed in Haiti starting in 1994, routine medical surveillance was carried out at 22 military clinics<sup>(52)</sup>. Among the 406 combat support hospital admissions during the first 6 months of deployment, 25% were due to febrile illnesses. More detailed diagnostic studies showed that dengue fever accounted for at least 30% of febrile illnesses leading to hospitalization. DEN-1, DEN-2 and DEN-4 were isolated from the troops. The clinicians observed that they were unable to diagnose dengue based on clinical grounds alone<sup>(53)</sup>.

## Clinical highlights

The clinical presentations of dengue in the Americas are generally similar to the cases in south-east Asia. However, the age distribution of DHF cases in the Americas differed somewhat from Asia. In Puerto Rico in 1990-1991 the mean age of patients who developed DHF was 38, whereas in south-east Asia primarily young children become ill with DHF<sup>(54)</sup>. In the 1994 epidemic of dengue fever and dengue haemorrhagic fever in Ceara State, Brazil, the mean age of DHF cases was 42<sup>(55)</sup>. In the 1990-91 study of 56 confirmed cases of DHF in Rio de Janeiro, Brazil, the modal age range was 31-45<sup>(56)</sup>. Likewise, in the 1997 outbreak in Cuba, DHF primarily affected young adults<sup>(33)</sup>. The overall case-fatality rate of DHF in the Americas from 1997-2002 ranges from 0.9% to 1.6%<sup>(24,57,58,59,60,61)</sup>.

## New approaches to surveillance

In Puerto Rico dengue is a well-known and common infection, and surveillance systems are in place. In contrast, leptospirosis is rarely reported, though a seroprevalence study found antibodies in 14% of the population. After Hurricane Hortense hit the island in 1996, resulting in heavy rains and flooding, leptospirosis was diagnosed in patients initially suspected to have dengue fever. This led investigators to do a study using an island-wide dengue laboratory-based surveillance system. Serum samples from patients with suspected dengue that tested negative for dengue were subsequently tested for the evidence of leptospirosis. They found that before the hurricane, 6% of the dengue-negative patients have had leptospirosis. This increased to 24% (17/70) during the post hurricane period<sup>(62)</sup>.

In French Guiana investigators tested a laboratory surveillance system to try to find a way to identify a dengue epidemic early in its course<sup>(63)</sup>. Because the area is endemic for malaria and malaria smears are typically included as part of the work up of a febrile patient, they monitored the number of studies for malaria that were negative for malaria as a way to capture non-specific febrile illnesses. In that setting, the number of negative malaria studies was found to be a good predictor of dengue fever in some towns. This underscores the need to have baseline information about diseases present in an area and to base surveillance systems on local or regional characteristics relevant to the population.

## Conclusions and recommendations

Dengue is spreading geographically in the Americas, reaching larger populations and causing a more severe disease in areas that have concurrent or sequential circulation of multiple serotypes. Sustained control of the vector is difficult to achieve. Programmes to date have had limited sustained success, except perhaps in focal areas.

Better rapid diagnostic tests are needed. Also needed are more complete epidemiological studies by geographical area which would provide a profile of common infections in each area that can resemble dengue fever. Experience gained from outbreak investigations and surveillance studies, as described above, has shown that diseases as diverse as leptospirosis,

Venezuelan haemorrhagic fever, influenza, rubella, measles, and rickettsial infections have been misdiagnosed as dengue fever, or vice versa. Mechanisms of transmission, approaches to treatment, and control measures vary substantially among these infections, making accurate and timely diagnoses essential to provide informed interventions.

Perhaps because dengue fever is often not diagnosed or reported, the impact of the disease has been underestimated. Given the current circumstances in the Americas (population location, density, mobility, living conditions and current status of vector distribution and density) and what is known about the complexity of dengue viruses and the immune response they induce, it is likely that dengue fever will grow in importance in the Americas in the coming decades.

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# Dengue in Latin America – A Unique Situation

by

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## Abstract

The incidence and distribution of dengue virus infection in the tropical regions of Central and South America, as well as the Caribbean, has dramatically increased during the last two decades as a consequence, among other factors, of a wider distribution of *Aedes aegypti*, higher population density in many large urban areas, lack of effective programmes to contain vector development and deterioration of the urban environment. Some distinctive epidemiological patterns of transmission, such as the occurrence of large epidemics of both dengue fever and dengue haemorrhagic fever separated by long periods of absence of viral circulation among the population, are seen in the area. Concurrent circulation of all four serotypes of the virus is not unusual, favouring the occurrence of more cases of secondary infections, and a higher risk of dengue haemorrhagic fever and dengue shock syndrome. Unlike other geographic regions, in the Americas older age groups are widely involved. Some clinical manifestations of dengue in adults appear to differ from those habitually described in children and unusual complications, such as acute acalculous cholecystitis and parotitis, have been described. Further clinical information needs to be generated on the impact of dengue virus co-infection with other endemic agents present in the area.

**Keywords:** DF/DHF, *Aedes aegypti*, deterioration, urban environment, acute acalculous cholecystitis, parotitis, Americas.

## Overview

Although known to cause large outbreaks in the Caribbean since the first half of the 17th century, and continental epidemics or true pandemics during the 19th and 20th centuries, dengue virus was isolated for the first time in the Americas in 1953<sup>(1,2)</sup>. The first large epidemic of dengue haemorrhagic fever (DHF) in the region occurred in Cuba in 1981, with 24,000 cases of DHF and 10,000 cases of dengue shock syndrome

(DSS) and 158 deaths reported during a 3-month period<sup>(1,2,3,4)</sup>. In 1986 and 1987 massive outbreaks of dengue fever (DF) were reported in Brazil<sup>(5,6)</sup>. Subsequent serological investigations in the same country estimated almost 4 million cases of DF compared with the clinically estimated 1 million<sup>(6)</sup>. In 1988, an outbreak of DF was reported at 1700 m above sea level in Guerrero State, Mexico<sup>(7)</sup>. In 1990, almost one-fourth of the 300,000 population of Iquitos, Peru, contracted DF<sup>(8)</sup>

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and in the same year, 3,108 cases of DHF with 78 deaths were reported in Venezuela<sup>(9)</sup>. The last available regional figures, corresponding to the year 2001, indicate the occurrence of 610,625 clinical cases, 15,376 of which were of DHF/DSS, with 92 deaths. Several Latin American countries report the concurrent circulation of all four serotypes of the virus<sup>(1)</sup>.

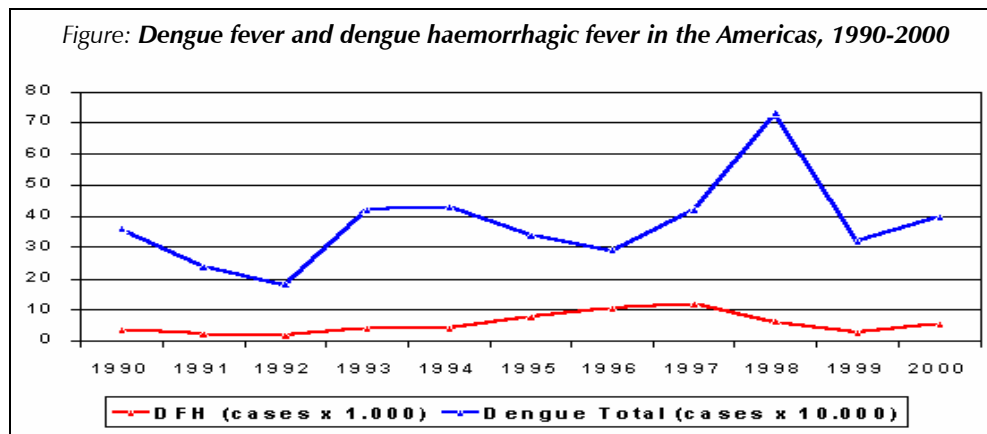
During the last decade, the spread of dengue fever was most dramatic in virtually all Latin American and Caribbean countries infested with *Aedes aegypti*. A sharp upward trend in the number of cases reported each year, from over 250,000 in the early 1990s to more than 600,000 by the end of the century, has been observed<sup>(2)</sup>. Furthermore, serological surveys suggest the occurrence of millions of such infections<sup>(10)</sup>. In the period between 1968 and 1980, only 60 cases of DHF from five countries, were reported in the entire region. However, after its emergence in Cuba in 1981<sup>(11)</sup>, epidemics or sporadic cases of dengue haemorrhagic fever (DHF) have been reported in at least 25

countries in the Americas<sup>(12)</sup>. Since 1989, when a large epidemic with 2,500 cases of DHF occurred, Venezuela has reported large numbers of DHF cases every year, and in 1995 the country recorded the largest regional outbreak with almost 30,000 dengue cases and 5,000 DHF cases. Although DEN-1, DEN-2 AND DEN-4 were isolated during this epidemic, DEN-2 was by large the most predominant serotype<sup>(13)</sup>.

## Regional epidemiology

Today, dengue and sometimes haemorrhagic dengue affects most of the American continent and several islands of the Caribbean. According to the Pan American Health Organization (PAHO), dengue transmission has increased significantly in the region in the last two decades (Figure) <sup>(1,4,14)</sup>.

Overall, between 1995 and 1997, the region experienced an annual increase in DF incidence rate of +12% and +35%, respectively, with a simultaneous increase in the DHF incidence rate of +61.87%<sup>(1,14,15)</sup>.



Source: PAHO Reports



By the year 2000, 27 countries of Central and South America, and the Caribbean, reported dengue transmission, of them, 17 registered cases of haemorrhagic dengue and 10 registered deaths by DHF. The most affected subregion was South America, and the hardest hit countries were Brazil, Ecuador, Colombia, Paraguay and Venezuela.

Major determinants for the occurrence of dengue in Latin America have been defined as follows<sup>(5,7,8)</sup>:

### **Population growth**

The percentage of urban population and the expansion of mega cities (10 million inhabitants or more) are on the increase. It has been estimated that by 2020, urban population in Latin America will be about 80% of the total (from 42% in 1954), and by 2030, close to 50% of the population will live in mega cities<sup>(9,15)</sup>. High population provides an opportunity for increased rates of dengue transmission.

### **Unplanned urbanization**

Unplanned urbanization is almost always accompanied by lack of civic amenities, inadequate and intermittent water supplies and poor solid waste disposal, and provides increased breeding sites for vector species.

### **Air travel**

Globalization of world economies and tourism have promoted increased travel for the people of the region to different parts of the world, including dengue endemic countries. This has facilitated importation of

DEN viruses into the region. Importation of south-east Asian strains of DEN-2 and Sri Lankan/Indian strain of DEN-3 are the classic examples. This virulent strain has been responsible for the higher number of DHF cases in the region.

### **Poor sanitary conditions**

As in other parts of the world, the main factors directly or indirectly influencing the magnitude of dengue transmission in Latin America appears to be the low socioeconomic levels of the population and poor sanitary conditions<sup>(2,11,12)</sup>. This has helped in the wider spread of *Aedes aegypti*.

### **Deterioration of the public health infrastructure**

Deficient regional public health systems, as well as an ineffectual and obsolete sanitary legislation, are hardly a match to the emergent DHF threat. Besides, due to economic constraints, health authorities are more inclined to take emergency contingency measures rather than undertake preventive activities on a regular basis.

### **Is clinical expression of the disease in the region any different?**

In contrast with observations in Asian countries, where DHF is almost completely restricted to young children, in the Americas, older age groups are widely involved<sup>(2,6,16,-27)</sup>. For example, during the Venezuelan outbreak of 1989, about one-third of the deaths were among patients over 14 years of

age, while in the 1997 Cuban outbreak, all of the deaths were among adults<sup>(19)</sup>. Moreover, in Puerto Rico, in 1990-91, the reported mean age of the patients was 38 years<sup>(20)</sup>, and during the 1981 Cuban outbreak of DHF/DSS, the frequency of DHF/DSS was higher among female adults<sup>(19)</sup>. Of late, an increase in the percentage of DHF cases in individuals over 15 years of age has been noticed in Malaysia and the Philippines in recent years<sup>(28)</sup>; however, young children continue to be the age group predominantly affected<sup>(26,28)</sup>.

Many potential factors may influence the type and severity of the disease arising out of any epidemic of dengue<sup>(20)</sup>. The host immune response appears to be a major factor. Sequential infection with different dengue viral serotypes in the presence of non-neutralizing antibodies has been strongly incriminated in the occurrence of DHF/DSS<sup>(19,20,21)</sup>, and cases of DHF/DSS are seldom documented in patients with primary infection<sup>(22,23,24,25)</sup>. Besides virus strain virulence, individual factors, such as age, sex, genetic background and underlying diseases, may also play a role<sup>(19,21,22,25)</sup>.

The severity of the dengue virus infection appears to be influenced by race. For instance, white individuals in Cuba were affected more significantly than blacks and mulattos by DHF/DSS in both the recent outbreaks<sup>(15,16,19)</sup>. Unlike most Latin American countries, Cuba has a predominance of whites, blacks and mulattos in its population, and lack Amerindians or mestizos, since the native population was completely exterminated during the colonial time.

As epidemics progress, some Latin American countries have recorded a significant steady increase in the proportion of total cases presenting as DHF or DHF/DSS, and in the case fatality rates for both DF and DHF/DSS<sup>(29)</sup>. Such increases have been explained by the fact that a part of the population with antibodies against a dengue virus serotype raised after natural prior primary infections would react with "neutralization" determinants found on a different serotype. These heterotypic antibodies would not prevent a secondary dengue infection, but would serve to down-regulate the disease to mild illness or symptomless infection. Nevertheless, a sub-population of the new viral serotype that replicates in the hosts immune to the preceding serotype might escape heterotypic neutralization. When inoculated into a new host immune to the prior serotype, these viruses would be free to interact with the more abundant infection-enhancing antibodies, thus producing severe disease<sup>(29)</sup>.

Adults seem less likely than children to suffer from DSS. Indeed, in a retrospective study of 108 adult Malaysians with DHF, the overall morbidity was significant (29.4%) but the case fatality rate remained low (2.0%). The lowest platelet level occurred on day 6 of the fever. Hyponatremia was observed in 46.8% of the cases<sup>(30)</sup>.

Some clinical manifestations of dengue in adults appear to differ from those usually described in children. For instance, several comparative studies have found hepatomegaly in only 10.5% of adults, as compared to more than 70% in children<sup>(31,32)</sup>.

Unlike children, adults with DHF/DSS often exhibit massive gastro intestinal or other sites bleeding, severe enough to cause death, prior to the onset of shock<sup>(32,33)</sup>. In fact, the latter is frequently a consequence of massive bleeding. Liver necrosis may be severe and has been observed in fatal cases among children and adults, both in primary and in secondary infections<sup>(20,21,34,35)</sup>.

Of note here is that about 10% of 97 Venezuelan adults with dengue recently studied by us (Torres JR et al. unpublished data) developed acute acalculous cholecystitis (AAC), according to clinical and ultrasonographic criteria. The latter included: enlarged gall-bladder with thickened wall ( $\geq 6$  mm) and pericholecystic fluid appearing as a halo, tenderness to palpation with the ultrasound probe, or the presence of a diffuse, homogeneous, non-shadowing, medium-level echogenicity within the gall-bladder lumen, were all considered "positive" findings<sup>(36)</sup>. Whereas AAC patients exhibited a statistically significant increase in the level of peripheral blood leukocytes, clinical outcome appears not to differ from that of patients without AAC in terms of length of clinical interval prior to admission and hospitalization, or the occurrence of other life-threatening complications. Details of our findings on this newly recognized condition will be discussed elsewhere.

Only scattered reports exist in the medical literature on the pathological and clinical implications of AAC complicating adults with DHF<sup>(37,38,39,40)</sup>. However, recent data in children with DHF suggest that a gall-bladder wall thickening  $\geq 5$  mm on ultrasonography correlates with a higher risk of hypovolemic shock.

The relatively common occurrence of dengue virus infection among adults in the region allows for the recognition of some complications of the disease harder to be noticed in affected children. This is the case of the recent description by us of acute bilateral parotitis<sup>(40)</sup>. More-over, clinical experience continues to accumulate on the impact of coinfection with dengue virus and other endemic agents present in the area, such as *Paracoccidioides braziliensis*, *Histoplasma capsulatum*, *Leishmania spp*, etc.<sup>(2)</sup>

In conclusion, DHF/DSS continues to occur in the Americas in a significant number of adults, but it is not clear whether this relates with the genetic background of the populations, epidemiological events, or else, with other unknown factors.

## Regional health impact and perspectives for control

Little information exists on the impact of dengue in the region in terms of the disease burden. Based on the experience in Puerto Rico, using disability-adjusted life years (DALYs) as a means of assessing the economic impact of dengue, dengue was found to cause the loss of an average of 658 DALYs per year per million population<sup>(41)</sup>. It has been estimated that the loss to dengue is similar to the losses per million population in the Latin American and Caribbean region attributed to any of the following diseases or disease clusters: the childhood cluster (polio, measles, pertussis, diphtheria, tetanus), meningitis, hepatitis, or malaria. The loss is also of the same magnitude as any one of the following: tuberculosis, sexually transmitted diseases (excluding human

immunodeficiency virus), tropical cluster (e.g. Chagas' disease, leishmaniasis), or intestinal helminths<sup>(41)</sup>. These results suggest that when resources for research and control are allocated regionally, dengue should be given a priority equal to many other infectious diseases that are generally considered more important.

The application of vector control methods, including source reduction, use of chemical larvicides and adulticides and of biological control agents, is hampered by weak programme capacity, the absence of well-defined indicators and programme targets, and poor understanding of the efficacy and cost-effectiveness of control measures, particularly in terms of reducing transmission. Major epidemiological and operational research challenges are a better understanding of the virus transmission dynamics and the identification of transmission thresholds<sup>(42)</sup>.

A key factor to be considered in any control programme requiring a strong social participation component is "behaviour change". As in other parts of the world, recent dengue prevention and control programmes in the Americas have relied

upon educational approaches, on the premise that knowledge would lead to behaviour change<sup>(12,16,42)</sup>. However, experience with this and similar programmes, such as HIV and diarrhoeal diseases prevention and control, have demonstrated that a poor correlation exists between knowledge improvement and behaviour change. Hence, emphasis must be shifted to the development of behaviour change interventions. For this purpose, ministries of health and communities need to develop stronger links both among themselves and with other key partners in order to achieve a sustainable reduction in the risk of infection and burden of disease.

PAHO reported that in 1995, only about US\$ 104,000,000 were spent on dengue control activities in the Americas<sup>(42)</sup>. This amount is clearly insufficient for the purpose. Therefore, unless significantly larger resources are allocated and more aggressive and effective vector control measures are carried out, the countries of the region will continue to face repeated epidemics of dengue, and, as a consequence, an increased danger of DHF epidemics.

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# Dengue in Brazil: Situation-2001 and Trends

by

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## Abstract

Successive epidemics of dengue have been occurring in Brazil since 1986 and almost three million cases of dengue fever (DF) and 2,229 cases of dengue haemorrhagic fever (DHF) had already been recorded till 15 September 2002. The introduction of the three serotypes in circulation (DEN-1, DEN-2 and DEN-3) has always started in Rio de Janeiro. Approximately 47,370 and 89,394 cases of dengue due to DEN-1 were recorded in 1986 and 1987 respectively, corresponding to a risk rate of 34.5 and 64.63 per 100,000 inhabitants. The two following years were characterized by a low occurrence of DF. The introduction of DEN-2 in 1990 was also followed by an epidemic reaching close to the magnitude of the previous epidemic (27.29 and 71.1 per 100,000 inhabitants in 1991 and 1992 respectively). From 1994 onwards, the transmission rapidly progressed to many Brazilian cities and this wave of epidemics remained constant for four consecutive years, reaching a peak in 1998 (326.4 cases per 100,000 inhabitants). However, it is very clear that the decline of this latest epidemic did not attain the inter-epidemic levels of the two previous waves, when the risk varied from 1.13 cases per 100,000 inhabitants in 1988 to 4.87 cases per 100,000 inhabitants in 1993, as the rate always remained greater than 127 cases per 100,000 inhabitants. The fourth wave began in 2001, shortly after the DEN-3 was detected, and was characterized by increased rates of both DF and DHF (2,669), considerably higher than the total accumulated over the entire previous decade (896).

**Keywords:** Dengue, epidemiology, trends, control strategies.

## Introduction

The re-emergence of infections of the dengue virus in Brazil has contributed to a sharp change in the tendency and pattern of morbidity, this disease being the most common of those making up the list of compulsory notifiable diseases in the country. Considering the whole picture, this could have serious repercussions in the

profile of mortality resulting from infectious diseases. At present, DF/DHF is one of the major concerns of the Brazilian public health authorities, but it affects urban centres, hitting the tourism industry. The situation is compounded by the inability of the local health governments to effectively control it.

Three serotypes of the virus (DEN-1, DEN-2 and DEN-3) have already been

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isolated in the country and there is an imminent risk of the introduction of DEN-4 due to the continual, intense air and sea traffic with other countries of the Americas and other dengue endemic continents (Table 1). This is likely to aggravate the current epidemiological scenario.

**Table 1:** Dengue serotypes isolated in the Americas, 1990-2001

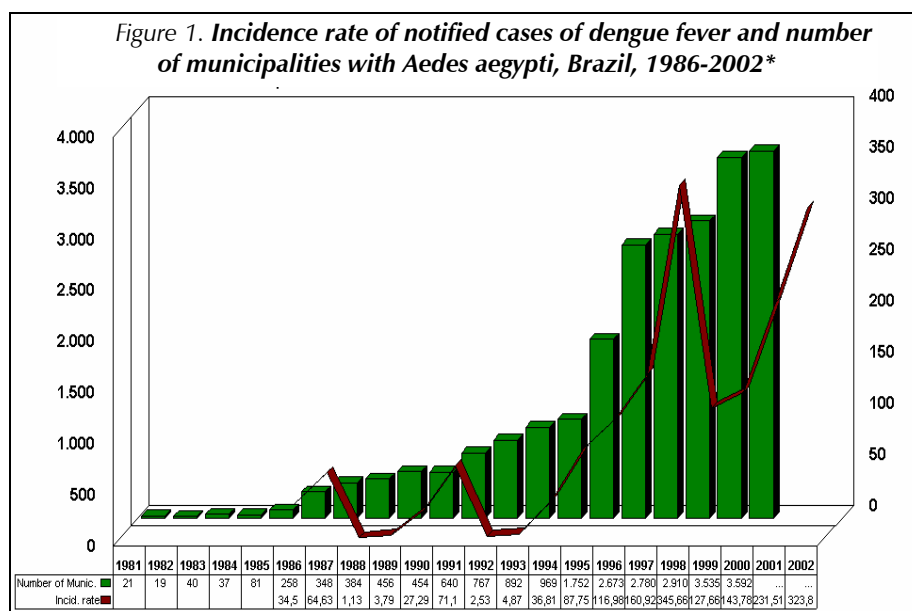
República Dominicana	DEN-1, DEN-2, DEN-3, DEN-4
El Salvador	DEN-1, DEN-2, DEN-3, DEN-4
Belize	DEN-1, DEN-3
Jamaica	DEN-1, DEN-3
Islas Virgins	DEN-1, DEN-4
Guadalupe	DEN-1, DEN-2, DEN-3, DEN-4
Martinica	DEN-1, DEN-2, DEN-3,
Barbados	DEN-1, DEN-2, DEN-3, DEN-4
S. Vicente & Granadinas	DEN-1, DEN-2, DEN-3
Dominica	DEN-1, DEN-2, DEN-3
Trinidad & Tobago	DEN-1, DEN-2, DEN-3, DEN-4
Suriname	DEN-1, DEN-2, DEN-3, DEN-4
Guiana Francesa	DEN-1, DEN-2, DEN-3, DEN-4
Porto Rico	DEN-1, DEN-2, DEN-3, DEN-4
Granada	DEN-2, DEN-3
Cuba	DEN-2, DEN-3, DEN-4
Nicaragua	DEN-1, DEN-2, DEN-3, DEN-4
Costa Rica –	DEN-1, DEN-2, DEN-3
Panamá	DEN-1, DEN-2, DEN-3, DEN-4
Venezuela	DEN-1, DEN-2, DEN-3, DEN-4
Ecuador	DEN-1, DEN-2, DEN-3, DEN-4
Brasil	DEN-1, DEN-2, DEN-3
Peru	DEN-1, DEN-2, DEN-3, DEN-4
Paraguay	DEN-1, DEN-2

## Current situation and tendency of circulation of dengue virus

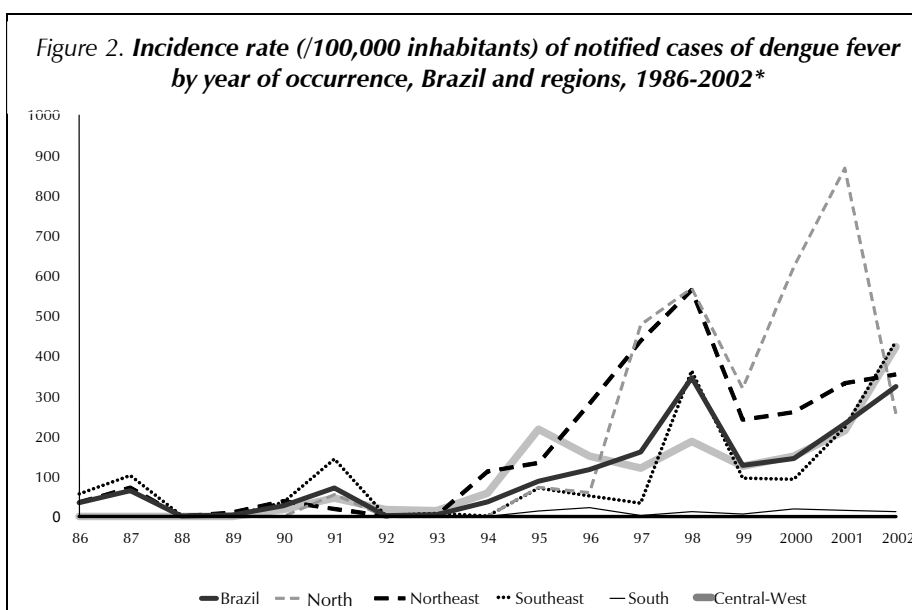
The circulation of the dengue virus in Brazil has been very intense since 1986 when the DEN-1 serotype was introduced into the country. This virus caused large epidemics of dengue fever in urban areas, i.e. the metropolitan region of Rio de Janeiro, the country's most important tourist centre. DEN-2 was isolated in 1990 when an explosive epidemic of dengue fever occurred and a few cases of dengue haemorrhagic fever were also registered.

Between 1986 and 1993 (Figures 1 and 2), two epidemic waves occurred, similarly characterized by two peaks in two consecutive years (maximum incidence of 66.1/100,000 inhabitants in 1991), followed by intervals of low incidence, which also lasted two years. From 1994, due to the spread of the virus to different regions, an exponential growth of cases began to be registered, reaching a maximum peak in 1998 (more than 570,000 cases reported) when the risk of becoming sick reached 345.7/100,000 inhabitants. At this time, the north-eastern region of the country became the area of highest risk (564.1/100,000 inhabitants in 1998) and also became the area with the largest number of recorded cases (258,441). In 1998, around 2,675 of the 5,507 municipalities in Brazil had already been affected by the DEN-1 and DEN-2 serotypes, and the *Aedes aegypti*, the only transmitter of dengue in the country, had been detected in 2,910 of these municipalities. In only two states in the south of Brazil, Santa Catarina and Rio Grande do Sul, where the climate is cold and inhospitable to the proliferation of the vector were there no autochthonous cases of the disease.





Source: Ministry of Health of Brazil \* Preliminary data up to May 30, 2002



Source: Ministry of Health, Brazil \* Preliminary data up to May 30, 2002

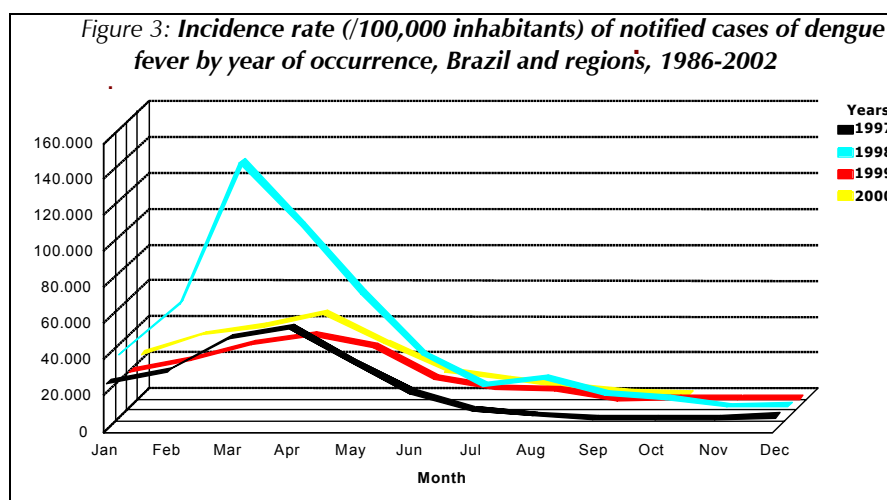
In 1999, the incidence of the disease expanded geographically to smaller cities, principally in the north, while the overall rate of infection simultaneously fell to 127.7/100,000 inhabitants, most likely due to the reduced number of susceptible individuals and partly due to the tightening of vector control activities.

Nevertheless, in December of the following year, DEN-3 was isolated for the first time in Rio de Janeiro. It was here that the fourth large dengue epidemic began, the rate of cases reaching 231.5/100,000 inhabitants in 2001. In the first five months of 2002, more than 550,000 cases of dengue fever were registered (323.8/100,000 inhabitants), and the risk of the occurrence of the disease is predicted to remain high in future as well.

It has thus been noted from the temporal tendency curve of dengue fever (Figure 1) that while the first two epidemic waves had similar forms and greatly-reduced inter-epidemic levels (between 1.1 and 4.9/100,000 inhabitants), the third epidemic showed longer progression, higher incidence and a much less

accentuated reduction in the occurrence level (greater than 127/100,000 inhabitants), far from the inter-epidemic levels of the two previous loops. After this, the introduction of the DEN-3 serotype led to the appearance of a fourth epidemic wave that, only 17 months after its appearance, seemed close to reaching the levels observed in 1998 when the disease attained its maximum peak, resulting in a situation of increased intensity and speed in the simultaneous transmission of the three serotypes, the principal predicting factor of explosive epidemics of DHF<sup>(1,2)</sup>.

The occurrence of dengue in Brazil is well defined by the seasons, the greater incidence occurring in the first months of the year, particularly between March and May (Figure 3) when the prevailing temperature and humidity conditions are conducive for the proliferation of *Aedes aegypti* in most of the country. No gender difference had been noted and the age group >15 years suffered most. However, it would seem that the predominance of the infection in this age group is falling, possibly due to the greater number of susceptible younger victims<sup>(3)</sup>.

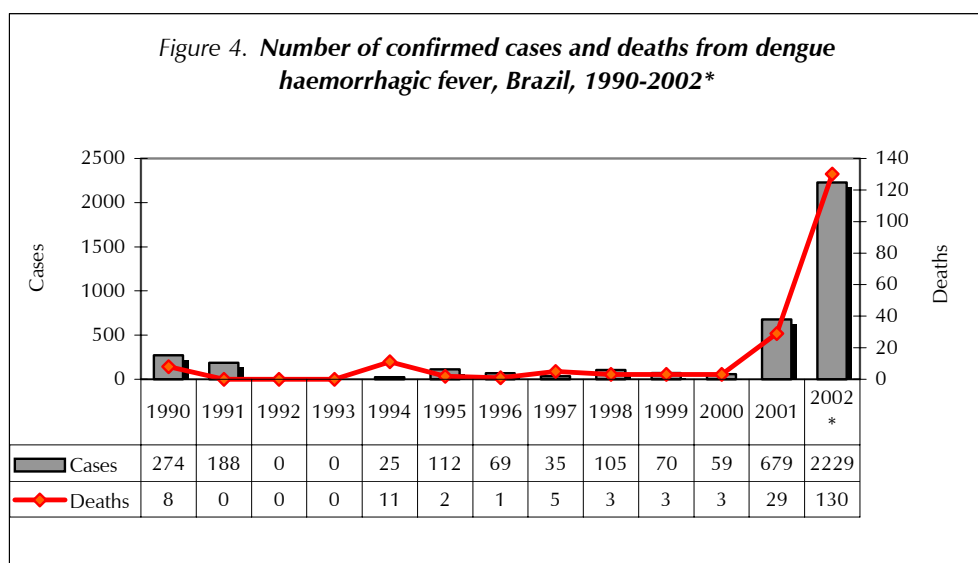


## Dengue haemorrhagic fever

Contrary to some other countries in the Americas such as Cuba<sup>(4)</sup> and Venezuela<sup>(5)</sup>, dengue epidemics in Brazil up to the year 2000 registered cases comprising of DF (almost two million cases) and low notifications for DHF (896 cases), despite the intense, simultaneous circulation of the two serotypes (DEN-1 and DEN-2). During 1990-2000, the case-fatality rate of DHF was relatively high, around 6%, when in some countries of south-east Asia, for example Thailand, this rate was lower than 1%<sup>(6)</sup>. In the two years of 1990 and 1991, in the state of Rio de Janeiro immediately following the introduction of the DEN-2 serotype, 1,316 cases were registered and 462 cases were confirmed as of DHF with eight deaths. In the following years, there were relatively few cases of DHF registered, the maximum annual number being 114 in 1995. Between

1990 and 2000, the total number of accumulated cases of DHF (944) corresponded to only 0.05% of the registered cases of the disease.

Due to the circulation of the DEN-1 and DEN-2 serotypes of the disease, and because of the estimates that millions of individuals already had antibodies to these serotypes as demonstrated by the high seroprevalence detected in serological investigations<sup>(7,8,9,10)</sup>, a higher rate of DHF was predicted. This fact was attributed in part to diagnostic difficulties as a consequence of deficiencies in the medical care system, and in part due to the rigorous criteria of the WHO, adopted by the Ministry of Health, for the confirmation of cases. In addition, a hypothesis has been raised of the reduced virulence of the strain of DEN-2 circulating in the Americas<sup>(11)</sup>.



Source: Ministry of Health, Brazil

\*Preliminary data up to 15 September 2002

Compared to previous patterns, the clinical expression of the disease was modified after the introduction of DEN-3, when the fourth epidemic wave occurred. The incidence of DHF increased because of exposure of the population to sequential infections. As expected, this latest epidemic which is still in course, showed a greater incidence and severity in the state of Rio de Janeiro where 1,728 of the 2,229 confirmed cases of DHF in the country have occurred till 15 September 2002. During January 2001 and May 2002 (Figure 4), more than 960,000 cases of DF were registered in the country and DHF represented almost 0.3% of the total. The mean case-fatality rate continued to be high at around 3.4%.

## Control strategies

Stimulated by the need to eliminate the circulation of the yellow fever virus from its urban centres, Brazil was one of the countries on the American continent that in the first half of the twentieth century developed intensive efforts to combat the *Aedes aegypti*, receiving certification of its eradication in 1958. This vector was sporadically detected after 1958; however entomological vigilance and quick implementation of effective control strategies resulted in complete elimination of *Aedes aegypti* from infested areas.

After 1976, the slackening of these measures in the country resulted in a gradual re-infestation by the species. In 1986, due to financial constraints and also due to the fact that some countries in the Americas had already been re-infested, the Pan-American Health Organization (PAHO) suggested switch over of the strategy from eradication

to that of simple control of the *Aedes aegypti*<sup>(11)</sup> vector.

Brazil continues to make great efforts to control this mosquito but, as in most other countries the impact, both on the reduction and expansion of the vector population and on the control of the circulation of the dengue virus, has been very limited<sup>(12)</sup>. In 1995, the Ministry of Health made an effort to return to the eradication strategy but due to political, administrative and financial difficulties, the plan, which had been drawn up, was never implemented<sup>(13)</sup>.

The current programme of dengue control is focused principally on the chemical control of the vector through the treatment of the foci with larvicides and the use of ultra low volume (ULV) insecticides. The other two components of this programme, environmental sanitation and health education actions, are also being developed, albeit on a reduced scale as compared to the country's actual requirements. In hundreds of Brazilian cities the infestation indicator most used by the programme, the Premise Index, remains on average above 5%, with great variations that sometimes reach levels above 10%.

It can clearly be seen in Figure 2 that the growth of the circulation of the dengue virus in Brazil followed the territorial expansion of its vector and that, despite annual spending of around half a billion dollars on vector control, there has been no success in the effective control of the disease.

## Challenges and perspectives

Even considering countries with well-structured programmes, the experiences in

vector control in general or eliminating the disease or preventing the introduction of new serotypes are not promising. Taking the current situation of the infestation by *Aedes aegypti* in Brazil into account, and the existence of a large population with antibodies to DEN-1 and DEN-2 and recently to DEN-3 serotypes, as well as the possibility of the introduction of DEN-4, the future looks dismal. New outbreaks/epidemics of dengue are expected in the coming summers, possibly with a growing

proportion of DHF cases as compared to previous years. Since the only alternative available for prevention is vector control, there is a need to adopt innovative community-based sustainable control strategies, based on environmental sanitation supported by effective health education programmes. Simultaneously, improvements in the case-management of individuals suffering from severe forms of the disease should be implemented with a view to reducing the case-fatality rate.

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# Dengue Viruses in Brazil

by

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## Abstract

Dengue was first recognized in Brazil in 1981-1982. However, the disease became a nationwide public health problem after epidemics occurred in the state of Rio de Janeiro which were caused by DEN-1 and DEN-2 in 1986 and 1990, respectively. Widespread circulation of viruses in the entire country resulted in about 75% of dengue cases notified in the Americas in the last 8 years, with an increased incidence of DHF/DSS. All Brazilian regions have been affected by the epidemics. However, the North-East and South-East have a higher number of notifications.

The more recent introduction of DEN-3 in the state of Rio de Janeiro resulted in the emergence of the largest epidemic with more than 220,000 notified cases of dengue between January-May 2002. The co-circulation of three dengue serotypes has been responsible for the increase in the severe forms of the disease i.e. DHF/DSS.

**Keywords:** Dengue/Dengue haemorrhagic fever spread, public health problem, epidemics, multivirus circulation, Brazil.

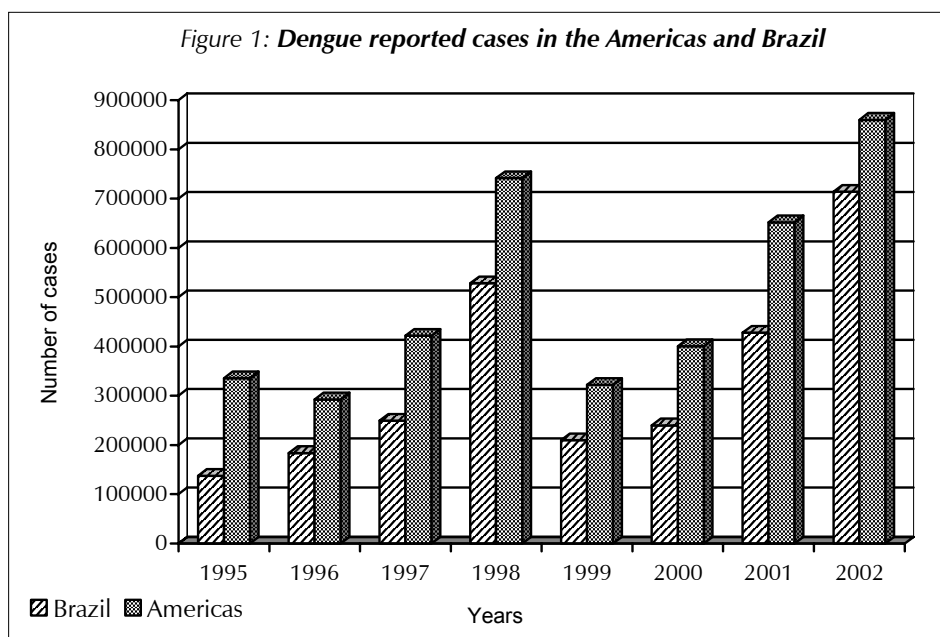
## Introduction

The high level of dengue virus activity in the American continent and the reinfestation of Brazil by *Aedes aegypti* in 1977 contributed to the reintroduction of the dengue (DEN) viruses into Brazil in the 80s<sup>(1,2)</sup>. From that decade onwards, the country has been responsible for more than 75% of the reported cases of dengue in the Americas (Figure 1).

A dengue outbreak due to DEN-1 and DEN-4 viruses (1981-1982) occurred in the city of Boa Vista, state of Roraima in the Amazon region<sup>(3)</sup>. This episode was controlled by local measures of vector elimination and no dengue activity was notified during the following four years in the country. It was only in 1986 with the introduction of the DEN-1 virus into the state of Rio de Janeiro that dengue became a nationwide public health problem<sup>(4)</sup>.

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Source: Americas data from [www.paho.org](http://www.paho.org)

Source of Brazilian data: National Centre for Epidemiology, Ministry of Health Brazil until 31<sup>st</sup> epidemiological week

Difficulties in implementing an effective vector control programme resulted in the rapid spread of the virus and consequent occurrence of epidemics in several states. The situation was aggravated in 1990 by the introduction of the DEN-2 virus into the state of Rio de Janeiro<sup>(5)</sup> and its subsequent spread to other regions in the country. By 2001, 25 of the 27 Brazilian states had reported dengue epidemics, with a total of 3 million cases, resulting from DEN-1 and DEN-2 epidemics in the last 16 years<sup>(6)</sup>.

After complete absence from the Americas for almost 15 years, the DEN-3 virus was reintroduced into the continent in 1994<sup>(7)</sup>, reaching Brazil in 2000 and causing a large and severe dengue epidemic in the summer of 2001-2002<sup>(8,9)</sup>. The state of Rio de Janeiro again proved to be the nodal state

for the introduction and dissemination of this new serotype in the country.

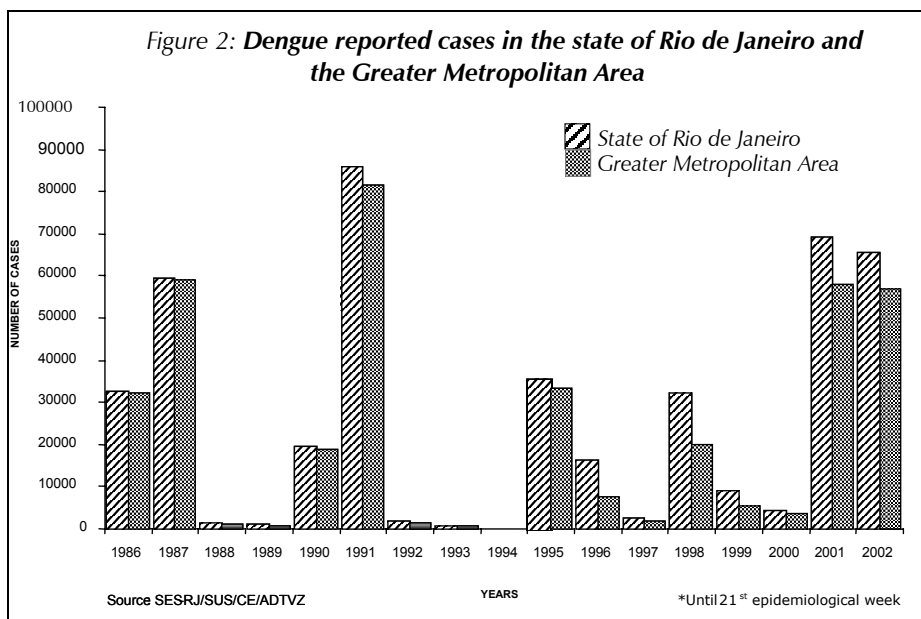
## Dengue in the state of Rio de Janeiro

The dengue infection was first confirmed by our laboratory in April 1986, when the DEN-1 virus was isolated from the blood of 8 cases (8/8), collected during an epidemic in the municipality of Nova Iguaçu<sup>(10)</sup>. This municipality belongs to the Greater Metropolitan Area of the state, which includes the capital, Rio de Janeiro, and 20 other municipalities, with 11,151,639 inhabitants out of the 14,768,969 inhabitants in the whole state. This heavy circulation of people in the region facilitated the rapid spread of dengue virus, causing an explosive epidemic, with 92,000 cases reported during the 1986-1987 period<sup>(11)</sup>.

An active surveillance programme by the Health Secretary of Niterói (Greater Metropolitan Area) resulted in the early identification of DEN-2 by April 1990, exactly four years after the DEN-1 virus isolation and during a period of high DEN-1 virus activity. During the epidemic in 1990-1991, which presented two waves, a significantly greater proportion of patients with thrombocytopenia and requiring hospitalization were seen in the DEN-2 predominant phase<sup>(5)</sup>.

Both the DEN-1 and DEN-2 viruses were isolated during a new epidemic recognized in 1995-1996, with a total of 51,465 reported cases of dengue fever. In January 1998, a new epidemic broke out in the Paraíba river valley and quickly spread to other municipalities in the state reaching an important tourist area on the northern coast of the state<sup>(11)</sup>.

During 2000, a Virological Surveillance Programme was carried out in Nova Iguaçu during an inter-epidemic period. This resulted in the isolation of the DEN-3 virus from a case with classical dengue fever and from the vector *Aedes aegypti* collected in the field<sup>(8,12)</sup>. The introduction of the DEN-3 virus increased the number of notified cases to 69,269 in 2001 and during the summer of 2002, caused the most severe epidemic in the state of Rio de Janeiro so far observed<sup>(9)</sup>. The number of reported cases exceeded the epidemic of 1990-1991 in which more than 100,000 cases, with 462 cases of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) and 8 deaths were reported. In 2002, until the 21st epidemiological week, a total of 224,684 cases had already been reported with 1,728 cases of DHF/DSS and 61 confirmed deaths. The higher notification of the disease was observed in the Greater Metropolitan Area which has been the most heavily affected area with respect to dengue outbreaks (Figure 2).





Laboratory studies carried out on 1,478 suspected dengue cases in the same period confirmed a 54.5% infection rate by serology and/or virus isolation and reverse transcriptase-polymerase chain reaction (RT-PCR). Three DEN-1, one DEN-2 and 320 DEN-3 virus strains were detected, showing that this new serotype represented 98.7% of the circulating viruses during the 2002 epidemic in the state. Forty fatal cases were confirmed in the laboratory, 20 of them were shown to be positive by at least two different methodologies, and the DEN-3 virus was the only serotype detected in these cases.

### DEN-1, DEN-2 and DEN-3 spread to Brazilian states

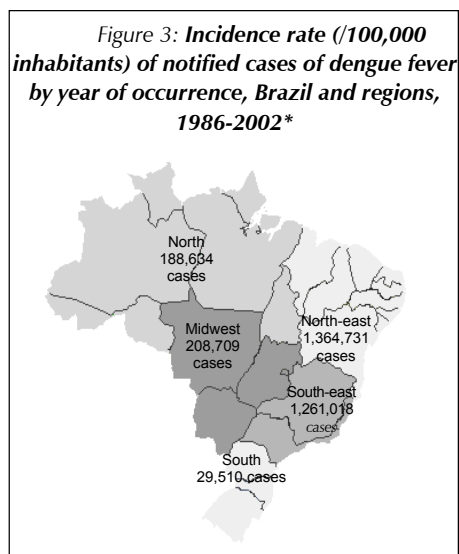
The South-East and the North-East are the most affected regions by dengue infections, with epidemics occurring almost yearly. In the South-East region besides the state of Rio de Janeiro, the states of Minas Gerais and Espírito Santo have also been reporting epidemics in both the capital cities and inland municipalities. In the state of São Paulo, dengue viruses activity is mainly observed in inland municipalities and sporadically in coastal localities. The North-eastern region, which is composed of nine states, has suffered successive dengue epidemics and it has been responsible for the highest number of dengue notifications during the later 90s<sup>(13,14,15,16,17)</sup>.

The Midwest region, which includes the Federal District, confirmed DEN-1 virus circulation in 1990. In 1995, DEN-2 virus was isolated and one case with dual infection was reported<sup>(6,18)</sup>.

In 1991, a dengue epidemic caused by the DEN-2 virus occurred in the state of Tocantins<sup>(19)</sup> and in 1995 in the state of Pará<sup>(20)</sup>, both in the Northern region. The state of Roraima confirmed dengue activity in 1996, 14 years after the first outbreak occurred in the state. In 1998, the state of Amazonas notified a dengue epidemic with 23,910 cases. In 2001 all states in that region, including Acre and Amapá, were affected by epidemics of variable magnitude<sup>(6)</sup>.

In the Southern region, the state of Parana had been the only one notifying dengue since 1995. No indigenous cases had been notified so far by the states of Santa Catarina and Rio Grande do Sul<sup>(6)</sup>.

Figure 3 shows the distribution of dengue reported cases, according to the Brazilian regions. It should be pointed out that dengue infections in the country, according to the available epidemiological data, are in general found in all age groups.



**Table:** Number of DHF/DSS and deaths reported in Brazil, 1990-2001

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
DHF	274	188	–	–	25	114	69	46	105	72	40	679
Deaths	8	–	–	–	11	2	1	9	10	3	3	29

Source: National Center of Epidemiology, Ministry of Health, Brazil

–= indicates no notified DHF/DSS and deaths

The co-circulation of DEN-1 and DEN-2 viruses in Brazil led to the appearance of DHF/DSS (Table), initially in Rio de Janeiro and afterwards in other states<sup>(5,21,22,23)</sup>. The increase in the number of more severe cases in the country, like in many others in the Americas, was coincidental with the introduction of the DEN-2 south-east Asian genotype into the continent<sup>(24)</sup>.

Analysis by the sequencing of the genome, performed on DEN-1 and DEN-2 viruses isolated in Brazil, identified the Caribbean and the south-east Asian genotypes for the DEN-1 and DEN-2 viruses, respectively<sup>(25,26,27,28)</sup>. The partial sequence of the junction from E/NS1 of DEN-2 Brazilian isolates, during 1990 to 2001, showed that this genotype is still the only one circulating in Brazil (data not published).

The DEN-3 virus genotype introduced into the continent has been associated with major DHF/DSS epidemics in Sri Lanka and India and with DHF/DSS cases and deaths in Mexico and Central American countries<sup>(29,30)</sup>. The molecular characterization of DEN-3 viruses isolated from autochthonous cases in Rio de Janeiro, by the sequencing of structural proteins, showed that our strain belonged to the same genotype<sup>(31)</sup>.

Unusual manifestations such as the involvement of the central nervous system were first reported during the 1986-1987 epidemic in Rio de Janeiro and later in different states, including one case in the state of Rio Grande do Norte, where immunohistochemistry detected dengue antigen in neurons<sup>(32,33,34,35)</sup>.

High levels of serum amino-transferases have also been observed. In the more severe cases, yellow fever infections were occasionally suspected; however, epidemiological investigations and laboratory results confirmed dengue infection in all these cases.

During the DEN-3 epidemic in Rio de Janeiro, we were able to detect by RT-PCR, viral RNA in cerebrospinal fluid, liver, brain, lung, spleen, and kidney from fatal cases.

In summary, the dengue viruses activity in Brazil during the past 16 years is demonstrated by the high number of notified cases and the number of states involved in the epidemics. The high dengue endemicity besides the co-circulation of three serotypes have been responsible for the increase of the severe forms of the disease such as DHF/DSS in the country.

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# Eco-epidemiological Factors Associated with Hyperendemic Dengue Haemorrhagic Fever in Maracay City, Venezuela

By

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## Abstract

Present research describes the establishment of dengue haemorrhagic fever (DHF) in Venezuela (1989), and dengue dynamics in Maracay city from 1993 and 2001. We also studied the relationships between DHF and weather variables, and explored the relationships between the disease and indicators of public services and *Aedes aegypti* pre-adult (House, Breteau Index) and adult (resting) parameters in neighbourhoods with no (apparent) dengue, low, and high dengue incidence/persistence. Our analysis suggests that DHF emerged and got established in Venezuela as a result of a combination of the introduction of new, more pathogenic strains of dengue, high and widespread adult mosquito populations resulting from inadequate public services, lack of effective vector control, and dengue hyperendemicity. DF and DHF were well correlated with rainfall and humidity; however, transmission continued during the distinct dry seasons, when breeding places generated by water-storing devices produced high adult densities of *Aedes aegypti*. DF and DHF were associated with the frequency and length of water-supply interruptions, mosquito adults per room, human population density, neighbourhood area, and with the persistence or history of dengue transmission in the locality. Even low dengue/persistence neighbourhoods showed deficiencies in public services and elevated adult mosquito densities, showing that the number of mosquitoes is not a limiting factor for dengue transmission in most of Maracay city.

**Keywords:** Dengue haemorrhagic fever, *Aedes aegypti*, ecology, Venezuela

## Introduction

The first epidemic of dengue haemorrhagic fever (DHF) in Venezuela occurred in Maracay city in 1989. Since then this disease

has become endemic/epidemic in the country<sup>(1,2,3,4,5)</sup>. For yet unknown reasons, Venezuela had accounted for most number of DHF cases (52.5%) and deaths (36%) in the American region until 1998<sup>(6)</sup>. It seems

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that the emergence of DHF in Venezuela was concurrent with the emergence of a new strain of DEN-2 from south-east Asia, the one that circulated in Cuba (1981) and Jamaica (1982)<sup>(7,8)</sup>. This newly-arrived strain of DEN-2 has been replacing the previously established, more benign strain<sup>(7)</sup>. Although the introduction of new virus strains could partly explain the appearance of DHF, it would be important to explain its higher prevalence in Venezuela.

One explanation could be the increased local abundance and geographical spread of *Aedes aegypti*, the main dengue vector<sup>(9)</sup>. Venezuela could be particularly vulnerable since most of the human population is concentrated in cities at low altitude along the Caribbean coast, where environmental conditions are conducive for *Aedes aegypti*<sup>(10)</sup>. A study of 30 coastal towns in Venezuela showed that insufficient piped water supply and deficiencies in garbage collection were significantly associated with high infestation levels of *Aedes aegypti*. Water storing containers, mainly metal drums (200 lt.), were the most common breeding sites<sup>(11)</sup>. Some traditional customs, such as the use of flower vases in cemeteries, also contribute to high *Aedes aegypti* growth within cities<sup>(12,13)</sup>. Studies on the population dynamics of *Aedes aegypti* in an urban centre under such conditions have demonstrated high densities of adult populations during each month of the year, despite the distinct and long dry season<sup>(14)</sup>.

Longitudinal studies undertaken in Maracay city during 1993-98 indicated that DF and DHF were present in each of the 8 municipalities and showed positive relationship with human population density.

In general, DF and DHF were highly correlated at the neighbourhood level (linear regression  $R^2 = 0.91$ ) with 3 (clinically diagnosed) DF cases for each case of DHF. At a finer scale, most neighbourhoods (84%) showed dengue cases during the period of the study, and 83.4% of those with DF also had DHF cases. An analysis of the consistency in temporal occurrence of dengue per neighbourhood was performed correlating cases of DF, and of DHF, between years, resulting in each correlation being significant<sup>(5)</sup>. These results implied that neighbourhoods producing large (or small) numbers of dengue cases in one year continued to do so in each of the years considered. This high temporal consistency was likely to be the result of hyperendemicity, where different serotypes alternated in time. It was also demonstrated that neighbourhoods showing longer dengue persistence (maximum number of consecutive months with cases) also had the highest DF and DHF incidence and prevalence, allowing us to stratify the city in: 56 neighbourhoods with no (apparent) dengue, 238 with low incidence/persistence (dengue persistence 1-5 months 1993-98), and 54 with high incidence/persistence (6-50 months). The high incidence/persistence neighbourhoods accounted for 70% of all cases reported during the study, but occupied only 34% of the city's land area. Those neighbourhoods included the most populated localities<sup>(5)</sup>.

In an attempt to understand the differential spatial occurrence of dengue in Maracay city, we tried to understand the relationship between the *Aedes aegypti* indices, water supply and interruptions and the dengue incidence in different

neighbourhoods. The study attempted to explain the progression of the disease in Maracay city from 1993 and 2001, and the relationship between weather variables and dengue.

## **Materials and methods**

Maracay city (10° 07' – 10° 20' N; 67° 24' – 67° 38' W; 436 m altitude) has 8 districts with an approximate total population of 1 million people (1998, National Census Bureau), distributed in 348 neighbourhoods occupying 109 km<sup>2</sup> land area. The mean annual precipitation, temperature and relative humidity were 1,044 mm, 25.4° C, and 75.4% respectively (1993–1999; Venezuelan Air Force). There is a rainy season from May to October, and a dry season from November to April.

Dengue data at the national level came from an integration of clinically reported cases (Ministry of Health, Pan American Health Organization (PAHO)). Dengue data for Maracay came from raw paper forms of the clinically diagnosed cases (State Health Department) that were reviewed and incorporated into a Geographical Information System<sup>(5)</sup>. Criteria for DF and DHF followed PAHO's case-definition<sup>(15)</sup>. *Aedes* indices (House, Breteau) were investigated in each of 100 houses from 59 neighbourhoods during the dry season and 104 neighbourhoods during the rainy season in 1998 and 1999. Adult *Aedes aegypti* were searched for in approximately 100 houses from 11 neighbourhoods in the dry seasons (n=1096 houses) and 29 neighbourhoods in the rainy season (n = 2466). Adults were collected from the main room of each house with a CDC backpack aspirator<sup>(16)</sup>. Questionnaires asking the households about

the quality of public services were applied in the same neighbourhoods as above. The questions required the households to rank the frequency of piped water supply interruptions (1=daily, 2=several times a week, 3=several times a month, 4=seldom), length of interruption in water supply (1=hours, 2=days, 3=weeks), and the frequency of house garbage collections (1=daily, 2=2 or 3 times a week, 3=once a week, 4= once every 2 weeks, 5=never).

## **Statistical methods**

Associations between variables were analysed with Pearson's correlation coefficients ( $\alpha=0.05$ ). Statistical comparisons of mean neighbourhood areas and inhabitants among levels of dengue incidence/persistence were made with Analysis of Variance (ANOVA;  $\alpha=0.05$ ). Ordinal variables (public service variates) between types of neighbourhoods were compared using Mann-Whitney tests ( $\alpha=0.05$ ). Means for metric variables with homogeneous variances were compared using Student tests ( $\alpha=0.05$ ) for independent samples.

## **Results**

### ***Dengue in Venezuela and Maracay City***

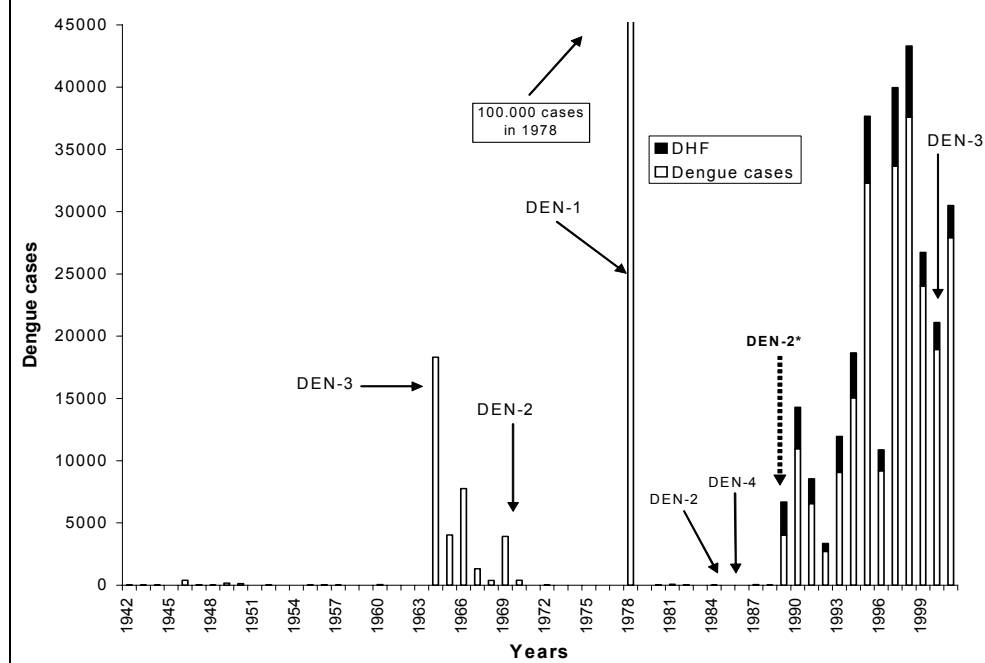
The reported dengue epidemics in Venezuela were relatively isolated in time and caused by single serotypes until the 1970s (hypoendemicity; Figure 1). The pattern suggests that the different dengue serotypes causing epidemics could not get established in the country, and that epidemics corresponded with dengue virus introductions. During 1989-2001, a total of

273,556 dengue cases were reported in Venezuela, of which 41,646 were DHF cases (15.2%). During the first DHF epidemic (1989-1990) in Maracay, the predominant dengue serotype was DEN-2, with the simultaneous circulation of DEN-1 and DEN-4<sup>(4)</sup>. Therefore, apart from the introduction of a more pathogenic DEN-2 strain at that time, there also was dengue hyperendemicity<sup>(7,8)</sup>. Therefore, it is apparent that since 1989 there have been DHF epidemics every year, and it has become endemic in large cities, such as Maracay (Figure 1).

DEN-3 appeared in Venezuela in September 2000 (D Tovar, National Institute of Hygiene, pers. com. 2001), and it was the

prevalent serotype circulating in Maracay during the epidemic of 2001 (G Comach, Regional Dengue Laboratory, pers. com. 2001). From 1993-2001 there have been 17,726 clinically diagnosed dengue cases in Maracay, of which 3,703 (21%) were DHF cases, with 13 deaths (0.35% case fatality rate). The yearly reported dengue cases fluctuated in time (713-4,597), and the highest values corresponded to the DEN-3 epidemic in 2001. The morbidity rates varied between 51 and 311 cases per 100,000 inhabitants, whereas the mortality rates changed from 0 to 0.37 per 100,000 inhabitants, without an apparent relationship between these two variables (Table 1). The case fatality rate varied between 0 and 0.38%.

**Figure 1: History of dengue epidemics in Venezuela 1942-2001. A South-East Asian DEN-2\* strain was associated with the emergence of dengue haemorrhagic fever in Venezuela**



Source : Ministry of Health, PAHO



### Dengue trends in Maracay

DF and DHF were reported during each month of the year, with peaks of varying intensity during the wet seasons since 1993 (Figure 2). It would seem as 3-year cycles of DF and DHF epidemics had been established in Maracay (e.g. lowest in 1996 and 1999). The introduction of DEN-3 by the end of 2000, and the epidemic caused by this serotype in 2001, seemed to have changed that temporal pattern. The years of lowest dengue (1996 and 1999) were preceded by years of peak dengue, and on each occasion, there was a delay in the appearance of dengue epidemics in the following year. For example, maximum dengue incidence was registered between July and August for most years, with the

exception of 1997 and 2000, when peak dengue showed up between October and November/December.

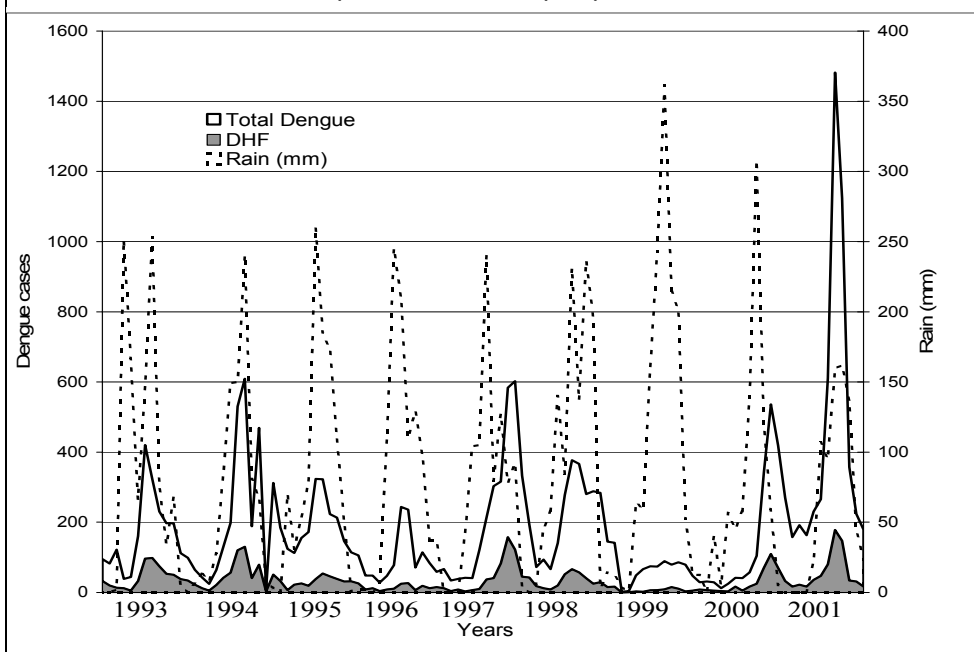
Peaks of DF and DHF cases were near concurrent with rain peaks, showing a significant correlation with the amount of rain ( $r=0.43$ ,  $P<0.05$ ;  $r=0.26$ ,  $P<0.05$ , respectively). In spite of the markedly seasonal pattern of dengue incidence in Maracay, dengue transmission continued during the dry seasons. Figures for dry season prevalence of total dengue (21.9%) and DHF cases (22.1%) were high during the study<sup>(5)</sup>. An example of the continuous dengue circulation during the dry season was the presence of dengue cases from December 2000 to April 2001 (Figure 2), when low rainfall was recorded in 5 months (5.6 mm).

**Table 1:** Clinically diagnosed DF and DHF in Maracay City, Venezuela, 1993–2001

Year	Total dengue	DHF	% DHF	Deaths	Morbidity rate per 100,000	Mortality rate per 100,000	Case fatality rate (%)
1993	1,495	528	35.3	0	125	0	0
1994	1,864	565	30.3	1	138	0.1	0.18
1995	2,006	398	19.8	5	149	0.37	1.25
1996	989	174	17.6	2	73	0.15	1.15
1997	2,165	530	24.5	2	155	0.15	0.38
1998	2,200	389	17.7	2	157	0.15	0.51
1999	713	79	11.1	0	51	0	0
2000	1,697	381	22.5	1	115	0.1	0.26
2001	4,597	659	14.3	0	311	0	0

Source: State Health Department

Figure 2: Clinically diagnosed cases of dengue fever and dengue haemorrhagic fever, and rainfall (mm) by month in Maracay City, Venezuela, 1993–2001



The temperature tends to increase and the relative humidity to decrease towards the end of the dry season, whereas relative humidity remains high during the rainy season and several weeks thereafter. DF showed a positive correlation with the relative humidity ( $r=0.40$ ,  $P<0.05$ ) and a negative correlation with the evaporation rate ( $r=-0.42$ ,  $P<0.05$ ). Despite good correlations with the weather, it seems other variables also influenced the annual patterns of dengue. For example, the low dengue incidence in 1996 and 1999 could not be explained by the lack of rain, but perhaps by the population herd immunity due to the prevailing serotypes causing large numbers of infections in the previous years. Also, the

major epidemic in 2001 caused by DEN-3 can be explained by the lack of population immunity, since this serotype had not been present in Venezuela since the 1960s.

### **Variables related to dengue transmission**

The DF and DHF incidence and persistence, population density and other variables were compared among neighbourhoods with (i) no apparent dengue (56 neighbourhoods), (ii) low (238) and (iii) high (54) incidence/persistence (Table 2). Statistical tests were performed mainly between the variables of low and high dengue neighbourhoods, because few neighbour-

hoods with no (apparent) cases of dengue were sampled. Neighbourhoods with higher DF and DHF incidence and persistence were more populated, occupying larger areas, and exhibited more water storage due to frequent and longer interruptions in piped water supply (Table 2). Despite the significant differences observed in the quality of water supply, the low incidence/persistence neighbourhoods also showed lesser water storage due to smaller interruptions in piped water supply.

*Aedes aegypti*'s indices (House Index, Breteau Index) in both dry and wet seasons were relatively high and similar in neighbourhoods with low and high dengue incidence/persistence. The most abundant breeding place of *Aedes aegypti* in Maracay was the metal drum used for water storage, followed by miscellaneous containers, discarded tyres and ornamental plants (Table

2). Despite the lack of significant differences between indices in both types of neighbourhoods, some breeding habitats were significantly more abundant in areas with higher dengue incidence, such as animal watering pans, water-storing tanks, and potted plants/flower vases (Table 2). Although metal drums used for water storing seemed to be more abundant in neighbourhoods with higher dengue incidence, the difference was not statistically significant. This can be explained by the low productivity of metal drums due to lack of food. The number of female adult *Aedes aegypti* captured in the main room of houses was significantly larger in neighbourhoods with higher dengue incidence/persistence (2.2 adults), although the vector was relatively abundant in neighbourhoods with low dengue incidence/persistence (1.5 adults).

**Table 2:** Annual DF and DHF incidence, and disease persistence (maximum number of consecutive months with cases) per neighbourhood in Maracay City, Venezuela (1993–2000)<sup>#</sup>

Dengue and related variables per neighbourhood (Mean/sample size)	Neighbourhoods stratified according to dengue incidence and persistence		
	No apparent dengue	Low	High
Total dengue incidence (cases)	0 (56)	15 (238)	155 (54)
DHF incidence (cases)	0 (56)	4 (238)	35 (54)
Total dengue per 100,000 inhabitants	0 (42)	977 (230)	1,895 (49)
DHF per 100,000 inhabitants	0 (42)	238 (230)	445 (49)
Dengue persistence (months)	0 (56)	<u>2</u> (238)	<u>12</u> (54)
Neighbourhood area (hectares)	25.4 (56)	<u>24.0</u> (238)	<u>69.3</u> (54)

Dengue and related variables per neighbourhood (Mean/sample size)	Neighbourhoods stratified according to dengue incidence and persistence		
	No apparent dengue	Low	High
Inhabitants	1,269 (42)	<u>3,658</u> (230)	<u>19,355</u> (49)
Frequency of water supply interruptions	2.4 (6)	<u>2.6</u> (82)	<u>2.0</u> (35)
Length of water supply interruptions	1.6 (6)	<u>1.8</u> (82)	<u>2.3</u> (35)
Frequency of garbage collection	2.3 (6)	2.1 (82)	2.1 (35)
House Index in dry season	24 (2)	21 (36)	20 (21)
House Index in rainy season	17 (5)	20 (67)	23 (32)
Breteau Index in dry season	32 (2)	32 (36)	31 (21)
Breteau Index in rainy season	27 (5)	30 (67)	38 (32)
Animal pans per 100 houses	1.6 (5)	<u>1.5</u> (67)	<u>2.6</u> (32)
Water tanks per 100 houses	1.4 (5)	<u>1.3</u> (67)	<u>2.8</u> (32)
Metal drums per 100 houses	10.8 (5)	12.4 (67)	14.9 (32)
Potted plants and flower vases per 100 houses	4.6 (5)	<u>3.0</u> (67)	<u>4.3</u> (32)
Old appliances per 100 houses	0 (5)	0.3 (67)	0.1 (32)
Containers in plants per 100 houses	0.2 (5)	0.1 (67)	0.2 (32)
Tires per 100 houses	1.2 (5)	4.6 (67)	5.7 (32)
Miscellaneous containers per 100 houses	7.4 (5)	7.0 (67)	4.5 (32)
<i>Aedes aegypti</i> females per room	N/A	<u>1.5</u> (20)	<u>2.2</u> (14)

# Statistical comparisons were made only between neighbourhood with low and high dengue incidence/persistence, since only a few neighbourhoods without apparent data were sampled. Underlined values were statistically significant (PC 0.05)

## Discussion

### *Emergence of DHF in Venezuela*

The establishment of DHF in Venezuela as an endemic/epidemic disease was associated with the introduction of more pathogenic dengue virus strains, hyperendemicity and uncontrolled *Aedes aegypti* populations, generated by rainfall and water-storing practices. Our results showed significant associations of DF and DHF incidence with deficiencies in water supply at the neighbourhood level in Maracay. This was in tune with previous studies in several Venezuelan urban centres<sup>(10,11)</sup>. Public service deficiencies are the likely structural and long-lasting problems of neighbourhoods. It is perhaps for this reason that we have found that neighbourhoods are consistent in their temporal dengue patterns. The same neighbourhoods producing large numbers of cases in one year continued to do so throughout the study period. Also, neighbourhoods producing large numbers of cases exhibited high degrees of dengue endemicity, as estimated by dengue persistence (maximum number of consecutive months a neighbourhood produced dengue cases)<sup>(5)</sup>. If these conditions get compounded with multivirus circulation then it is not surprising to find endemic DHF.

### *Temporal dengue patterns in Maracay City*

The data showed good correlations between dengue and the meteorological variables associated with the ecological dynamics of *Aedes aegypti*. Positive correlations with rain

should reflect a larger abundance of aquatic habitats for immature mosquitoes; however, in this study larval indices did not differ between seasons. Entomological data for Maracay city shows that mosquito productivity is associated with ornamental containers, miscellaneous containers, metal drums, tyres, and animal pans. However, the frequency with which miscellaneous containers are found with pupae is higher than in any other container, which in turn may be responsible for a larger adult population during the rainy season. It is also likely that a higher humidity during the rainy season could influence vectorial capacity due to increased longevity. The large number of breeding places of *Aedes aegypti* existing during the dry season in Maracay may account for sustained dengue transmission, resulting in DF and DHF endemicity in the city.

Apart from the seasonal influence of weather on dengue incidence, yearly variations indicated the need to understand the dynamics of population immunity to each dengue serotype in Maracay. The lowest yearly dengue incidence rates were observed in the years immediately after a dengue peak, but the 2-3 month delay in outbreaks in the following year could be due to the exhaustion of susceptibles to the prevailing serotype(s). On the other hand, the large epidemic (2001) observed after the entrance of DEN-3 may also reflect the lack of immunity to this serotype that had been absent in Venezuela since the 1960s.

### ***Environmental variables and dengue***

Neighbourhoods with higher dengue incidence/persistence were characterized as having a larger human population, larger area, more accentuated deficiencies in the frequency and length of water supply interruptions, and more *Aedes aegypti* female adults resting inside the main room of houses (Table 2). However, neighbourhoods with lower dengue incidence/persistence exhibited some degree of deficiency of public services and relatively high *Aedes aegypti* densities (1.5 *Aedes* females per main room of houses). Such vector density is higher than that observed during some dengue epidemics (one *Aedes* female per house)<sup>(17,18)</sup>. If we consider that neighbourhoods in Maracay are not all isolated from each other, and that viruses could be frequently moved between neighbourhoods, the low incidence/persistence of dengue in those neighbourhoods seems to point out that other variables may be determining the level and constancy of dengue transmission. In other words, *Aedes aegypti* infestation is generally high and does not seem to be a limiting factor for dengue transmission in Maracay. Within the limitations of this study, the other significant variables separating low from high-dengue neighbourhoods were the number of inhabitants and the area, which should be correlated (Table 2). It is not surprising to find this result since there probably is a population density threshold for dengue transmission<sup>(17)</sup>. More populated neighbourhoods could also imply a more frequent virus exchange with other infected

neighbourhoods or cities, particularly in those including the main administrative and commercial centres of the city, and satellite towns of the Metropolitan Area of Maracay.

### ***Implications for dengue control***

Dengue control in Maracay city has been limited to emergency measures, such as outdoor spatial spraying of malathion (ultra low volume) from trucks around the block of notified dengue cases, campaigns for source reduction, and media efforts to obtain community involvement during epidemics. As the results of the temporal analysis showed, dengue persists as an endemic disease, and large epidemics resulting from the introduction of DEN-3 in 2000 could not be prevented with those control measures. Therefore, unless a change in the prevention/control approach takes place it is easy to predict the continued occurrence of endemic/epidemic DF and DHF in Maracay city.

Water storing due to deficiencies in piped-water supply is perhaps the greatest threat, followed by the widespread occurrence of miscellaneous, disposable containers, and the habit of keeping plants and flowers in water. Dengue control measures should be oriented towards drastic and permanent reductions in the number of containers holding water inside and outside buildings. A great deal of the solution to this problem could come from improving the water supply and garbage collection infrastructure, although it still would be necessary to modify behaviours such as those related to the use of plants and flowers

in water (gardens, inside the house, cemeteries, etc.), animal watering pans, maintenance of roof gutters, etc. Those tasks could only be accomplished with an adequate infrastructure and personnel for mosquito control directed towards the sustained reduction of breeding places.

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# How Effectively is Epidemiological Surveillance Used for Dengue Programme Planning and Epidemic Response?

by  
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## Abstract

Most dengue-endemic countries classify dengue/dengue haemorrhagic fever (DF/DHF) as a high priority disease in their planning and response documents. Very few of them, however, allocate the resources required to deal with DF/DHF as a high priority disease. A review of the surveillance activities in dengue-endemic countries and how surveillance data are used for planning and response revealed that few of them had effective surveillance systems for DF/DHF, and even fewer used available surveillance data in an effective manner for planning and response. The surveillance systems in selected countries with good surveillance are reviewed here. Issues of active vs passive surveillance and case definitions for DF and DHF are discussed, and recommendations made to improve the use of surveillance for planning and response.

**Keywords:** Dengue/dengue haemorrhagic fever, surveillance, epidemiology, epidemic response.

## Introduction

Epidemic dengue fever/dengue haemorrhagic fever (DF/DHF) has emerged as a major global public health problem in the past 20 years, with an increased incidence of the disease and expanding geographical distribution of both the viruses and mosquito vectors<sup>(1-2)</sup>. Factors responsible for this dramatic resurgence in the waning years of the 20th century are primarily demographic, societal and technical changes that have

occurred in the past 50 years<sup>(1-5)</sup>. Prospects are that the changes responsible for increased epidemic disease will continue indefinitely in future. Thus, effective prevention and control strategies are essential if we want to reverse the trend of more frequent and larger epidemics of DF/DHF.

Surveillance is an important component of any prevention and control programme<sup>(6)</sup>. Unfortunately, most dengue endemic

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countries have neither an effective surveillance system nor an effective mosquito control programme. The answer to the question, "How effectively is epidemiological surveillance used for programme planning and epidemic response?" therefore is that it is not very effective! Most DF/DHF endemic countries acknowledge the need for surveillance of this disease, but few of them have functional systems that can provide the support for programme planning, let alone for epidemic prediction and response. The table below lists the major DF/DHF endemic countries in

the world with a subjective evaluation of the status and efficiency of their surveillance systems, whether they have laboratory capabilities and whether their systems have an early warning predictive capability for epidemic transmission, something that is required if emergency response is to be effective. It will be noted that most of the 50 countries listed have a passive surveillance system, but few have the active, laboratory-based surveillance needed to predict epidemic DF/DHF. Scanning the table gives the impression that surveillance for DF/DHF is poor at best in most endemic countries.

**Table:** Major dengue/dengue haemorrhagic fever endemic countries and their surveillance capabilities\*

WHO Region/Country	Surveillance			Lab capability		Epidemic prediction
	Passive		Active			
	DF	DHF	DF/DHF	Serology	Virology	
South-East Asia						
Bangladesh	-	++	-	+	+	-
India	-	+	-	+	+	-
Indonesia	-	+++	-	+	++	-
Maldives	-	++	-	-	-	-
Myanmar	-	++	-	+	+	-
Sri Lanka	-	++	-	+	+	-
Thailand	-	+++	-	++	++**	-
Western Pacific						
Australia	++	++	++	+++	+++	-
Cambodia	+	++	-	+	+	-
China	-	+	-	+	+	-
Laos	+	+	-	-	-	-
Malaysia	++	+++	+	+++	+++	+
New Caledonia	++	++	-	+++	+++	-

WHO Region/Country	Surveillance			Lab capability		Epidemic prediction
	Passive		Active			
	DF	DHF	DF/DHF	Serology	Virology	
Australia (North Queensland)	+++	+++	+	+++	+++	+
Other South and Central Pacific Islands	+	+	-	-	-	-
Philippines	-	+	-	+	+	-
Singapore	+++	+++	+	+++	+++	+
Tahiti	++	++	-	+++	+++	-
Viet Nam	-	++	-	++	++	-
<b>Americas</b>						
Argentina	+	+	-	++	++	-
Barbados	+	+	-	+	-	-
Belize	+	+	-	-	-	-
Bolivia	+	+	-	+	+	-
Brazil	++	++	+	+++	+++	+
Colombia	+	+	-	++	++	-
Costa Rica	+	+	-	++	++	-
Cuba	++	++	-	+++	+++	-
Dominican Republic	+	+	-	+	+	-
Ecuador	+	+	-	+	+	-
El Salvador	+	+	-	+	+	-
French Guiana	+	+	-	+	+	-
Grenada	+	+	-	+	+	-
Guatemala	+	+	-	+	+	-
Haiti	-	-	-	-	-	-
Honduras	+	+	-	+	+	-
Jamaica	+	+	-	+	-	-
Lesser Antilles	+	+	-	-	-	-
Mexico	++	++	-	++	++	-
Nicaragua	+	+	-	++	++	-

WHO Region/Country	Surveillance			Lab capability		Epidemic prediction
	Passive		Active			
	DF	DHF	DF/DHF	Serology	Virology	
Panama	+	+	-	+	+	-
Paraguay	+	+	-	-	-	-
Peru	+	+	-	+++	+++	-
Puerto Rico	++	++	-**	+	-**	-
Suriname	+	+	-	-	-	-
Trinidad	+	+	-	-**	-**	-
United States	+	+	-	+++	+++	-
Uruguay	-	-	-	-	-	-
Venezuela	++	++	-	++	++	-
<i>African/Eastern Mediterranean</i>						
Djibouti	-	-	-	-	-	-
Eritrea	-	-	-	-	-	-
Kenya	-	-	-	+	+	-
Nigeria	-	-	-	-	-	-
Other African Countries	-	-	-	-	-	-
Pakistan	-	-	-	-	-	-
Saudi Arabia	+	+	-	+	+	-
Senegal	+	-	-	+	+	-
Somalia	-	-	-	-	-	-
<i>Others</i>						
Taiwan	+++	+++	-	+++	+++	-

\*The efficacy of the surveillance system and laboratory capability is rated as follows:

- surveillance or capability does not exist in public health laboratory

+ exists      ++ good      +++ best

\*\*Does not include US Military, CDC or WHO laboratories

There are two problems with passive surveillance for DF/DHF as it is conducted by WHO Member countries. First, there is no consistency in reporting standards. Some countries report only DHF while others report both DF and DHF. Secondly, the WHO case definitions are not strictly

adhered to in reporting the cases. These problems, which lead to both under-reporting and over-reporting<sup>(4)</sup>, must be corrected if we ever hope to obtain accurate incidence data on DF/DHF.

Most cases of DHF are reported in Asian countries; yet only two countries

(Malaysia and Singapore) have the laboratory capacity and actually support an active, laboratory-based surveillance programme, with the aim of predicting epidemic activity in advance of peak transmission. Several Asian countries have reasonably good passive surveillance systems for DHF (Sri Lanka, Myanmar, Thailand, Indonesia, Maldives, Malaysia, Singapore and Viet Nam). Very few of these countries, with the exception of Singapore, however, have a passive surveillance system for dengue fever, which, along with mild non-specific illness, is probably responsible for most transmission.

In the Americas, where DHF has emerged in the past 20 years, passive surveillance for dengue fever is better, since both DF and DHF are reported. Also, a number of countries, including the United States, Brazil, Puerto Rico and Cuba, have good laboratory capability to support active surveillance, and a number of other countries are developing that capability. Unfortunately, few countries support an active surveillance system with an early warning capability; only Puerto Rico has such a system that has accurately predicted three recent epidemics. Even in Puerto Rico, however, surveillance data from the active surveillance system have not been used effectively in emergency response to prevent the epidemics that were predicted.

In the Pacific, only Australia, Tahiti and New Caledonia have good laboratory capability, but it is not used to support active surveillance. And, in the African and Eastern Mediterranean countries, surveillance for DF/DHF is generally very poor.

In summary, the majority of dengue endemic countries do not have adequate

laboratory-based, active surveillance systems that can provide accurate early warning predictive capability for epidemic DF/DHF. And, those few countries that do have this capability, do not use it effectively for planning and emergency response. Most countries in dengue endemic areas usually do not respond to an epidemic until it is at, or near, peak transmission<sup>(4)</sup>. By then, it is too late for mosquito control measures to have any impact on transmission, even if they were effective. What characterizes DF/DHF surveillance in most countries is under-reporting during inter-epidemic periods and over-reporting during epidemics<sup>(4,6)</sup>. This crisis mentality must be changed if we ever hope to effectively respond to such epidemics by initiating prevention and control measures before epidemic transmission begins.

## **Country examples of DF/DHF surveillance**

I will use examples from some of the countries that I feel have the best surveillance systems, to illustrate what can be done, given adequate political and economic support, and some of the problems associated with these programmes.

### ***Singapore***

There is probably only one endemic country where surveillance is effectively used for planning, response and prevention and control; that country is Singapore<sup>(7)</sup>. Singapore uses case definitions and has mandatory reporting for both dengue fever and dengue haemorrhagic fever. Health authorities use the surveillance information to actively target specific areas of the city for

intensified control while maintaining a countrywide prevention and control programme.

The increased epidemic dengue activity in Singapore in the past 10 years is somewhat of a paradox, since the *Aedes aegypti* house indices have been held below 2% for a number of years<sup>(7)</sup>. The situation in Singapore underscores the need for regional prevention and control of this disease. The effective prevention and control programme in Singapore between 1968 and 1988 decreased the herd immunity to dengue viruses to all-time low levels<sup>(7,8)</sup>. The latter part of this period in the 1980s, however, coincided with a dramatic geographical spread and increased incidence of DF/DHF in most other surrounding countries of the Asia/Pacific region<sup>(2)</sup>. Increased disease incidence in those countries resulted in increased movement of dengue viruses. The combination of low herd immunity with increased importation of dengue viruses into Singapore, led to increased autochthonous transmission even though the *Aedes aegypti* population densities remained low (<2% house index)<sup>(9)</sup>. It should be noted that *Aedes albopictus* is also common in Singapore and may contribute to the transmission and maintenance of dengue viruses. However, this species is not an efficient epidemic vector of dengue viruses under most circumstances. It is likely that Singapore would not have epidemic DF/DHF if importation of the viruses could be prevented.

### **Puerto Rico**

Puerto Rico has one of the best, if not the best, surveillance systems for DF/DHF in the world<sup>(6,10-12)</sup>. The passive surveillance system

is based on case reports from physicians, clinics and hospitals from all over the island, and has been very effective as a result of intensive education programmes for both the public and the medical community. Both dengue fever and DHF are monitored, and clinical samples are submitted with the report. The active surveillance system is supported by the U.S. Centers for Disease Control and Prevention (CDC) Dengue Branch laboratory, and relies on testing blood samples submitted as part of the passive system. Selected cases, which are prioritized according to geographical area on the island and representative clinical severity, are processed weekly for dengue-specific IgM antibody and for virus isolation. The system is designed to provide real-time information on which serotypes of the viruses are being transmitted, where on the island the transmission is occurring, the severity of illness associated with each serotype, when a new serotype and/or genotype of the virus is introduced, and whether another flavivirus such as yellow fever or West Nile is introduced<sup>(10,12)</sup>. Reports are made to the Puerto Rico Health Department and back to the submitting physicians/clinics/hospitals on a weekly basis. The system is fully computerized.

Predictive capability for epidemic dengue transmission in Puerto Rico is based on the collective results of several types of data, including the number of case reports, seasonality, the IgM seropositivity rate, the severity of illness, the predominant virus serotype and strain isolated, and the geographical distribution of the laboratory-confirmed cases and the virus serotypes and strains isolated. This information is collected

and reviewed weekly, and over time, allows public health epidemiologists and laboratorians to gain “a feel” for dengue transmission in their catchment area, providing them with the real-time information they need to detect small changes that may be important. Of note here is the fact that mosquito surveillance is not considered a predictive factor in Puerto Rico because densities are high enough at all times of the year to transmit an epidemic<sup>(4)</sup>. Using this system, the last three major epidemics (1986, 1994 and 1998) were correctly predicted, with weeks to months as lead time before peak transmission occurred<sup>(13,14, CDC, unpublished data)</sup>.

Unfortunately, predictions are not always correct. A south-east Asian strain of DEN-3 was recently introduced in Puerto Rico, the first transmission of this serotype in 21 years. Based on this information it was anticipated that another epidemic would occur in 1999. For reasons that are not fully understood, this did not happen. Even so, the record of being correct 93% of the time (1 mistake in 18 years) is an excellent track record for predicting epidemic activity.

The objective of having an early warning surveillance system that can predict epidemics is to allow health authorities to implement early emergency response to reduce epidemic transmission, and save lives<sup>(1,4,6,10,12)</sup>. A major problem in Puerto Rico (and in most other endemic countries) is that the public health response to the surveillance data that are gathered in an active system is inadequate<sup>(4)</sup>. Thus, in both 1986 and 1994, when epidemics were predicted in June and July respectively, serious epidemic response measures were

not implemented until the peak transmission had occurred 3 to 4 months later. This late response is always too late to have any impact on epidemic transmission<sup>(4)</sup>. The third epidemic in 1998, however, was predicted in July and Puerto Rico health authorities responded early with apparent success. The surveillance data were discussed openly in the early stages of the epidemic, when increased transmission was just beginning, and an island-wide community-based source reduction campaign was initiated in July 1998. Although a proper evaluation of this campaign was interrupted by Hurricane Georges in late September, the case report data suggest that the epidemic had begun to wane in late August, 4-8 weeks before the expected peak of the epidemic based on historical data (CDC, Puerto Rico Department of Health, unpublished data). This experience leads us to believe that with active surveillance and early warning, major epidemics can be prevented using community-based larval mosquito control.

### **Cuba**

Cuba initiated a highly successful *Aedes aegypti* control programme during and after the 1981 DHF epidemic in that country, developing the best *Aedes aegypti* control programme in the region, with a house index of this species of less than 0.01% for over 16 years<sup>(15)</sup>. As a result, Cuba had no epidemics of DF/DHF through the 1980s and most of the 1990s. In mid-1990s, however, the mosquito surveillance and control programme had some problems. *Aedes albopictus* was introduced to the island and *Aedes aegypti* densities increased in some areas<sup>(16)</sup>. Although this problem was identified in late 1996, and cases were

reported as early as January 1997, an epidemic of DF/DHF occurred in Santiago de Cuba in June/July 1997<sup>(15)</sup>. Since that time, several outbreaks have occurred on the island. The Cuba experience once again underscores the old adage that "success breeds failure", and that, in the absence of total elimination, control pressure must be kept on *Aedes aegypti* in order to prevent a recurrence of epidemic transmission.

### **Brazil**

Brazil has an excellent laboratory-based surveillance system for DF/DHF, with a network of laboratories to conduct serological diagnosis of DF/DHF, and at least three laboratories to conduct virological surveillance. To my knowledge, however, the system is not used as an early warning system to predict epidemics. For example, in December 2001, DEN-3 was detected in Rio de Janeiro<sup>(17)</sup>, and despite warnings, control was not implemented until the epidemic was near its peak transmission. With appropriate coordination and data sharing, this system could become proactive and provide early warning for epidemic activity.

### **Malaysia**

Malaysia has a good laboratory-based surveillance system, with both serological and virological capability. However, it is basically a passive system and has little predictive capability unless a new serotype is detected.

### **Thailand**

Thailand has an excellent passive surveillance system for DHF, but does not

routinely report dengue fever, and has little laboratory support for areas outside of Bangkok. The Bangkok catchment area is served by the Queen Sirikit National Institute of Child Health (Children's Hospital), and the excellent laboratory support available from the Armed Forces Research Institute of Medical Science (AFRIMS), but this coverage is very limited. The rest of the country, where the majority of cases have occurred in recent years, has only a passive reporting system with no laboratory support. Plans are being developed to utilize selected provincial public health laboratories to develop a laboratory-based active surveillance system.

### **Other countries**

There are several other countries that are in various stages of developing a laboratory-based, active surveillance system for DF/DHF, including Indonesia, Viet Nam, Cambodia, the Philippines, India, Mexico, Venezuela, Peru and others in both Asia and the Americas. There are a few countries that are apparently serious about DF/DHF surveillance, but most do not provide adequate support and training to achieve this goal.

### **Emergency response**

In the early 1970s, decisions were made to move away from disease prevention programmes and place the public health emphasis on emergency response. This, in my view, was a disastrous public health decision. Public health policy- and decision-makers have since developed a "crisis mentality"; we wait until an epidemic is in progress before we respond and attempt to



control it. Unfortunately, passive surveillance systems are insensitive and rarely detect an epidemic much before peak transmission<sup>(4)</sup>. By then it is too late and a lot of money is wasted controlling an epidemic that is already waning. Equally unfortunate is that these epidemics receive a lot of coverage in the mass media and funds are generously allocated to develop an emergency response. The public and political outpouring of support during the period of crisis is gratifying to public health officials, but only serves to perpetuate the vicious cycle of epidemics and emergency response. This is not good public health practice when epidemic DF/DHF can be effectively and economically prevented.

There have been some attempts, mainly by the Pan American Health Organization (PAHO) to develop and initiate an early response to incipient epidemics in the region. An example was in 1994 when DEN-3 was isolated from patients in Nicaragua during a DF/DHF epidemic. The virus was shown to be an Asian strain closely related to the virus that caused recent DHF epidemics in Sri Lanka and India<sup>(18)</sup>. PAHO, working in collaboration with CDC, developed an emergency response plan and put out an alert urging countries in the region to enhance surveillance and implement preventive *Aedes aegypti* control. PAHO followed it up by helping countries in the American Region develop DF/DHF prevention and control programmes. Unfortunately, none of the American countries took this alert seriously, and DEN-3 subsequently spread rapidly, causing major epidemics throughout Central America and Mexico in 1995-1996<sup>(19)</sup>, and in Brazil in 2002<sup>(20)</sup>.

Similarly, in 1997-1998 in Asia, a number of countries experienced large epidemics (Indonesia, the Philippines, Viet Nam, Cambodia, Thailand, Malaysia and Singapore), despite efforts by the South-East Asian and Western Pacific Regional Offices of WHO to alert countries, urging them to develop prevention and control programmes (WHO, unpublished data).

## **Conclusion**

So "how effectively is DF/DHF surveillance used for planning an emergency response?" The answer has to be, "not very effectively!"

Dengue is a disease that is easy to overlook because the majority of infections are clinically non-specific and are usually diagnosed by physicians as something else during inter-epidemic periods<sup>(4)</sup>. Only when an epidemic occurs is the full spectrum of the disease reported, and then it is probably over-reported. Thus, the disease is under-reported during inter-epidemic periods, but rapidly gets over-reported when epidemic transmission is recognized. How do we solve this problem? The only solution is to implement standardized passive and active surveillance systems in all dengue endemic countries of the world, with emphasis on an early warning laboratory-based component<sup>(1,4,6,10,12)</sup>. Equally or even more important, however, is to educate policy- and decision-makers on effective use of active surveillance data to prevent major DF/DHF epidemics. Decision-makers must understand that to prevent epidemics a decision to respond must be made in the early stages when the transmission first begins to increase<sup>(4,12)</sup>.

WHO has already taken the first step in this direction by developing an electronic reporting system (DengueNet), recommending that both dengue fever and DHF be made notifiable diseases in endemic countries, by developing standardized case definitions for surveillance purposes, and by publishing guidelines for prevention and control<sup>(21)</sup>. Clearly, every dengue endemic country will need to develop and support laboratory diagnosis for DF/DHF to support active surveillance system<sup>(22-24)</sup>. Moreover, these countries will need to support the development and implementation of national prevention and control programmes if we ever hope to reverse the trend of increased epidemic DF/DHF<sup>(1)</sup>.

## Recommendations

1. All dengue endemic countries should develop and implement passive surveillance systems for dengue fever and dengue haemorrhagic fever using standardized case definitions developed by WHO.
2. All dengue endemic countries should develop and implement an active, laboratory-based surveillance system that is adapted to local conditions.
3. Regional reference laboratories should be developed and adequately supported to provide reference service and standardized reagents to national

laboratories. These centres should be provided the equipment and staff required to conduct a state-of-the-art laboratory diagnostic service.

4. Standardized reporting requirements, using DengueNet, should be developed and implemented in all endemic countries.
5. The DengueNet system should be used as a real-time international information exchange system so that endemic countries can share surveillance information on a timely basis with each other and with WHO.
6. Emphasis should be placed on programmes to educate physicians, nurses and others in the medical community in dengue endemic countries about DF/DHF, its diagnosis, management, prevention and control.
7. Every country should be encouraged to develop, implement and support national programmes for the prevention and control of epidemic DF/DHF, following the WHO Global Strategy.
8. Each WHO regional office should develop an emergency response plan that can be effectively implemented at appropriate times such as when major regional pandemics occur, as in 1998, or if a new virus such as yellow fever is introduced into the region.

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# Serodiagnosis of Dengue Infection by Rapid Immunochromatography Test in a Hospital Setting in Delhi, India, 1999-2001

by  
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## Abstract

A hospital-based cross-sectional serodiagnostic survey was undertaken during 1999-2001 at the Lok Nayak Hospital, Delhi, using a rapid immunochromatography test aimed at detecting anti-dengue antibodies from cases experiencing a febrile illness consistent with dengue infection. Acute-phase blood samples were collected from 345 patients attending outpatient and inpatient departments with clinical suspicion of dengue infection. A total of 85 patients were found to be antibody positive, of which 15 had IgM antibodies alone, indicating primary infection, whereas 19 cases had both IgM and IgG antibodies indicating secondary infection. The remaining 51 cases were presumed to have either secondary dengue infection or cross reactivity with other flaviviruses. Males outnumbered females during this study. Adults between the ages 21-30 years were found to be the most vulnerable group as 32 (37.6%) positive cases belonged to this group. Eighty-two per cent of positive serum samples were collected between October and November, thus indicating that the post-monsoon period was the most affected. Although the number of the serologically-confirmed cases of dengue infection had decreased, more community awareness and stringent measures for vector control are desired to contain this infection.

**Keywords:** Serodiagnosis, immunochromatography, dengue, Delhi.

## Introduction

Dengue infection is caused by any one of the four closely related but antigenetically distinct serotypes of dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4). In the past 60 years, dengue transmission and the

frequency of epidemics have increased dramatically in most tropical and subtropical countries, including the Americas<sup>(1,2)</sup>. This disease is more prevalent now than it was before and it is predicted to increase further<sup>(3)</sup>. In India, clinical disease compatible with dengue fever is known to

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have existed in an endemic form for a very long time. More than 50 outbreaks were reported during 1956-1996. All the four serotypes of dengue are prevalent almost all over the country. Due to its potential of rapid spread, dengue infection has now become a leading public health problem in India and has been declared as a notifiable disease in Delhi. Trends of the outbreak over the last few years indicate that dengue infection is now occurring with increasing frequency not only in urban areas but in rural as well<sup>(4,5,6)</sup>. Even so, it should be emphasized that a probable under-reporting of this disease occurs as a large proportion of the cases are asymptomatic.

A number of dengue outbreaks have been reported from the National Capital Territory of Delhi since 1967 and a major outbreak of DHF was reported in 1996. In this outbreak more than 10,000 cases and 423 deaths were reported, giving a case fatality of 4.1%<sup>(7)</sup>. After this outbreak guidelines were developed for the prevention and control of dengue by the Municipal Corporation of Delhi. Although due to these efforts dengue infection is under check in Delhi, however a few cases are still reported every year.

Serology is the mainstay of the diagnosis of dengue infection in most routine laboratories as it is rapid and easier to perform as compared to the conventional cell culture technique. Detection of antibodies is useful for the serological diagnosis of dengue, active surveillance and disease control. The present report on a hospital-based cross-sectional serological survey aimed at detecting anti-dengue antibodies from cases experiencing a febrile

illness consistent with dengue infection was conducted in Delhi during 1999-2001. The findings are presented below.

## **Methodology**

### ***Study design, population and sample size***

Acute-phase blood samples were collected from 345 patients experiencing a febrile illness consistent with dengue infection, attending the outpatient and inpatient departments of Lok Nayak Hospital, a tertiary care hospital in Delhi, over the period of three years. The study population comprised individuals of all age groups, selected according to the following inclusion and exclusion criteria.

### ***Case-inclusion criteria***

A case was included if there was high fever with clinical symptoms suggestive of dengue infection as per WHO criteria<sup>(8)</sup>.

### ***Case-exclusion criteria***

A case was excluded, if routine laboratory testing suggested bacterial or any viral infection other than dengue infection or any other disease.

A performa containing detailed clinical history and clinical findings was maintained for every patient.

## **Serology**

Dengue Duo IgM and IgG Rapid Strip test (Pan Bio, Australia) was used for the detection of anti-dengue antibodies. The

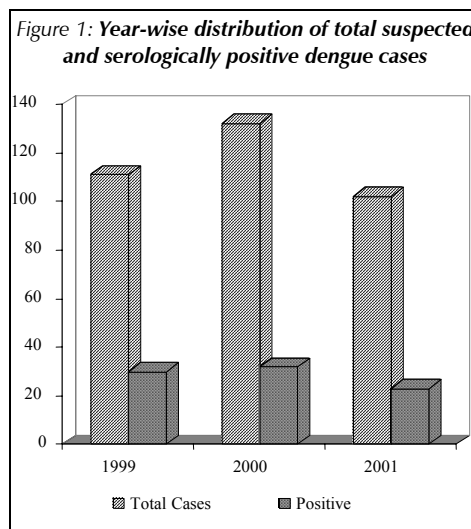
presence of IgM antibodies alone indicated primary infection whereas IgG and IgM antibodies indicated secondary infection. IgG antibodies alone was considered as suspected secondary infection or having cross-reactivity with other flaviviruses.

## Results

### Seropositivity

Blood samples were collected from 345 patients experiencing a febrile episode consistent with dengue infection over a period of three years, 1999-2001. Eighty-five cases (25%) were confirmed as serologically positive, with 15 cases showing IgM antibodies indicating primary infection and 19 cases showing both IgM and IgG antibodies indicating secondary infection (Figure 1). IgG antibodies alone were detected in 51 cases and these cases were presumed to have either suspected secondary dengue infection or had cross reactivity with any other flaviviruses. The year-wise case distribution revealed that in 1999, 27%, in 2000, 24% and in 2001,

22.5% suspected cases were serologically positive (Table 1).



### Distribution by age and sex

The maximum number of positive cases (32 out of 85) belonged to the age group 21-30 years; and males outnumbered females. In 1999, 40%, in 2000, 37.5% and in 2001, 34.8% of the cases belonged to the age group 21-30 years (Table 2).

**Table1:** Year-wise distribution of suspected and serologically positive dengue cases

Year	Total cases	Serologically positive cases	Number of cases with anti-dengue antibodies		
			IgM	IgM+IgG	IgG
1999	111	30 (27%)	4 (13%)	12 (40%)	14 (47%)
2000	132	32 (24%)	6 (18.8%)	3 (9.4%)	23 (71.8%)
2001	102	23 (22.5%)	5 (22.2%)	4 (17%)	14 (61%)
Total	345	85 (25%)	15 (17.6%)	19 (22.4%)	51 (60%)

**Table 2:** Age-wise distribution of serologically positive dengue cases

Year	Total positive cases	Age groups (years)			
		0-10	11-20	21-30	> 30
1999	30	4 (13.3%)	8 (26.7%)	12 (40%)	6 (20%)
2000	32	3 (9.4%)	10 (31.2%)	12 (37.5%)	7 (21.9%)
2001	25	6 (26.1%)	5 (21.7%)	8 (34.8%)	4 (17.4%)
Total	85	13 (15.3%)	23 (27.1%)	32 (37.6%)	17 (20)

**Table 3:** Month-wise distribution of serologically positive dengue cases

Year	August	September	October	November	December	Total cases
1999	0	2	15	12	1	30
2000	0	3	9	20	0	32
2001	1	4	3	11	4	23
Total	1	9	27	43	5	85

### Distribution by month and season

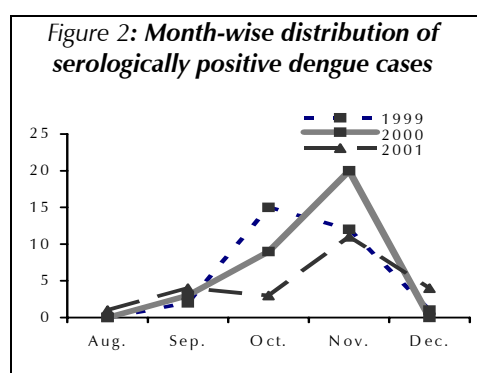
The maximum number of cases, 70 out of 85 serologically positive cases, were reported during October – November (Table 3). In 1999, the maximum number of cases (50%) were reported in October. During 2000 and 2001, the maximum number of cases (62.5%) and (48%) respectively, were reported in November. Thus, the seasonal occurrence of positive cases showed that the post-monsoon period was the most affected period, though the dengue vector is present throughout the year (Figure 2).

### Discussion

The analysis of the results in this study were based upon the interpretations mentioned in PanBio Rapid Immunochromatography test procedure manual. This rapid test had a good sensitivity and specificity comparable to other assays as reported by others and us in our previous studies<sup>(9,10)</sup>. Results were available within 30 minutes, and even with a single serum sample both IgM and IgG antibodies could be detected.

A total of 85 out of 345 suspected cases were found to be serologically positive

during the three-year study period. The positive cases included primary, secondary and suspected secondary infections. There was a slight increase in the number of cases with primary infection over the three-year period, though this was not significant. Sharma et al<sup>(11)</sup>, in their study in 1998, reported 64% cases with recent infection, which was higher than that observed by us. This was because our study was limited to only one hospital whereas they had cases from all over Delhi, or it suggested that there was a reduction in the cases with current infection. As compared to 1999, more than



a four-fold decrease was observed in the number of cases with secondary infection in 2000, while the decrease was more than two-fold in 2001 (Table 1). The total number of cases with dengue infection showed a declining trend during 1999-2001. A similar declining pattern in dengue infection was reported in Delhi during 1997-1999<sup>(12)</sup>. The most vulnerable age group was found to be 21-30 years (37.6%). Males outnumbered

females in all the age groups studied. Sharma et al<sup>(11)</sup> had reported that the maximum number of patients (69.5%) belonged to age 14 years and above. Quang et al<sup>(13)</sup> from north Viet Nam also observed the highest number of positives in the >20-year age group. These findings led to the suggestion that adults are at a higher risk of contracting dengue infection.

*Ae. aegypti*, the vector responsible for dengue infection, is present throughout the year in Delhi, but the seasonal peak of dengue infection coincides with the monsoon season, as stagnant water provides the most suitable conditions for its breeding<sup>(14)</sup>. In our study the post-monsoon period seemed to be the most favorable since 82% of the samples were collected during the months of October and November. A similar pattern was observed in 1996 and 1998 in Delhi<sup>(10,11)</sup>, Bangladesh<sup>(15)</sup> and Taiwan<sup>(16)</sup>.

Thus, it may be concluded from our study that there has been a decrease in the number of cases with dengue infection over the three-year period though fresh cases are being reported. As it may take years before a dengue vaccine is made available, measures to be considered for its containment are: improved sanitary conditions, community awareness and their participation to check and eliminate all kinds of breeding sites for the vector, and last but not the least, early serological detection of cases for active surveillance and disease control.

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# Study of Dengue Virus Infection in Kuwait

by

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## Abstract

Kuwait has a workforce of more than one million non-Kuwaitis coming from countries where vector-borne diseases like dengue fever are endemic. This study was aimed at investigating the magnitude of the dengue problem by determining the prevalence of dengue virus-specific antibodies and diagnosing suspected dengue fever cases in Kuwait. The antibody prevalence was determined by testing 909 serum samples from persons of various nationalities resident in Kuwait. In addition, laboratory diagnosis of the dengue virus infection was attempted on samples from 210 patients with the clinical presentation of dengue-like illness. Overall, 48% (436/909) donors had dengue virus-specific IgG antibodies. However, in Kuwaitis the seroprevalence was only 14% (59/425), which was significantly lower than in any group of the non-Kuwaiti residents, ( $p < 0.001$ ). Among the IgG-positive donors, the antibodies were most frequently detected against DEN-1 (55%), followed by DEN-2 (35%) and DEN-3 (10%). None of the 436 positive samples showed IgG antibodies to DEN-4. Moreover, among the 210 clinically suspected patients, IgM detection and RT-PCR confirmed dengue illness was found in 2 patients. Both of these patients were infected with DEN-2 and had visited dengue-endemic areas in the recent past. There was no evidence of the transmission of dengue infection within Kuwait as all of the 47 subjects who had never left Kuwait were found negative for dengue virus antibodies. The absence of *Aedes aegypti* in Kuwait and the poor vectorial capacity of *Aedes caspius* appear to be responsible for the absence of local transmission of dengue virus.

**Keywords:** Dengue fever, DEN-1, DEN-2, DEN-3, *Aedes caspius*, Kuwait..

## Introduction

Dengue virus belongs to the flaviviridae family and has four antigenetically but distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). Infection with any of these viruses is usually asymptomatic or produces

a mild, self-limiting disease, dengue fever (DF). However, in a small percentage of cases, the infection may result in a life-threatening syndrome, the so-called dengue haemorrhagic fever (DHF)<sup>(1,2)</sup>. The disease is reported in over 100 tropical and subtropical countries with about 2.5 billion people at

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risk of infection with dengue virus, leading to approximately 100 million cases of DF, 500,000 cases of DHF and 25,000 deaths per year<sup>(3)</sup>.

Kuwait is a small country with a typical desert climate located between latitudes 28° 35' and 30° 05' north of the Equator. The country has about 2.2 million people, out of which about 70% are non-Kuwaitis belonging to the expatriate workforce coming from countries where the dengue disease is endemic. Moreover, a large number of Kuwaiti nationals go on vacation to dengue hyperendemic areas during the peak season of dengue virus activity there. Therefore, there are ample opportunities of the virus infestation into the country by both expatriates as well as Kuwaiti nationals, as observed in other countries<sup>(4,5,6,7)</sup>

In view of the large-scale movement of the expatriate population and that of Kuwaiti nationals, there was a need to assess the potential of the dengue virus importation and the risk of establishment of dengue in Kuwait.

## Materials and methods

### Study population

The study was carried out between 1997 and 1999. Serum samples from age- and sex-matched donors were obtained from Kuwaiti nationals (n=425), individuals born and living in Kuwait permanently (Bedouins, n=47), and expatriates from South Asia (India, Pakistan, Sri Lanka, Bangladesh, n=266), south-east Asia (Philippines, n=31) and Middle East (Syria, Egypt and Lebanon, n=140). In addition, sera were obtained from a group of 210 patients admitted to the

Infectious Diseases Hospital, Kuwait. The patients were selected on the basis of clinical presentations compatible with dengue-like illness and also with febrile illness of unknown origin. All of them had recently come back after visiting dengue-endemic countries. Blood samples were collected from the patients on days 5-6 of the illness. The sera were frozen at -80°C until tested.

### IgG ELISA and blot tests

IgG ELISA was performed by using dengue virus (DEN 1-4) – specific antigens, control antigen and appropriate serum controls obtained from the Centers for Disease Control (CDC) Atlanta, USA. The ELISA procedure described by Gentry et al.<sup>(8)</sup> was followed.

IgG blot test was performed by using commercial kits (Genlab Diagnostics, Singapore) according to the manufacturer's recommendations.

In order to validate the serological survey, all the samples (n=909) were tested by both ELISA-IgG and dot blot-IgG tests. Sera being positive in both tests were considered as "true" positive for the presence of dengue-IgG antibodies.

### IgM capture ELISA

The test was done using plates coated with anti-human IgM antibodies according to the instructions of the manufacturer (PanBio, Australia).

### RT-PCR

The serum samples positive for dengue virus-specific IgM antibodies were screened for

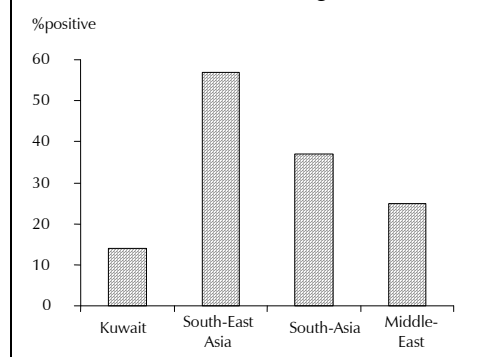
the presence of dengue virus (DEN 1-4) – specific RNA by an RT-PCR described previously<sup>(9)</sup>.

## Results

### ***Prevalence of dengue virus antibodies in people resident in Kuwait***

The findings (Figure) showed that maximum seropositivity was seen in subjects originating from south-east Asia (56.6%), followed by South Asia (37%), Middle East (25%) and Kuwait (14%). The 47 serum samples collected from Kuwaiti Bedouins, who were born in Kuwait and had never left the country, were negative for dengue IgG antibodies.

**Figure. Prevalence of dengue virus-specific IgG antibodies in sera from people originating from Kuwait and other regions**



The seroprevalence in the Kuwaitis was significantly lower ( $P < 0.001$ ) than in any group of expatriates. The serological data were further analysed to determine the serotypes of dengue viruses infecting the population in Kuwait. Among the 436

subjects found positive for dengue virus IgG, the most prevalent antibody was against DEN-1 (55%), followed by DEN-2 (35%) and DEN-3 (15%). None of the sera was positive for IgG antibodies against DEN-4.

### ***Laboratory diagnosis of dengue virus infection in clinically suspected patients***

The sera of 210 patients who had dengue-like illness were tested first for the presence of dengue virus-specific IgM antibodies. The capture IgM ELISA showed that 19 (9%) of them had dengue-specific IgM, thus suggesting recent infection with dengue virus. Among these 19 patients, 12 were Kuwaitis and 7 non-Kuwaitis. All of them had visited dengue endemic areas for 1 to 9 weeks and the time gap between the return to Kuwait and the onset of illness was from 2 to 10 days. The presence of dengue virus RNA was studied in the sera of all the 19 patients by RT-PCR using consensus primers, followed by semi-nested PCR with type-specific primers. RT-PCR was found positive for dengue virus in 2 patients and, in both of them, the semi-nested PCR identified the amplified products as DEN-2. One of these patients was a 30-year-old male Kuwaiti who had visited the Philippines for 5 weeks. He developed the clinical symptoms 7 days after his return and was sick for 10 days. The other patient was a 23-year-old Indian who had visited his country for 3 weeks and had developed the clinical symptoms 2 days after his return to Kuwait and was sick for 7 days.

## Discussion

This study was carried out to determine the magnitude of the threat of dengue to

Kuwait. The results of this study showed that the expatriates who come from dengue-endemic areas have a higher percentage of antibodies to the virus than the Kuwaitis. Although a fraction of the Kuwaiti nationals (14%) also had antibodies to the dengue viruses, the residents of Kuwait who had never left the country did not have antibodies against the dengue viruses. The magnitude of the seroprevalence of dengue in Kuwaitis found in this study was similar to what had been reported by Al-Nakib et al.<sup>(10)</sup>.

These results also suggest that despite the large number of the workforce coming to Kuwait from dengue-endemic areas, the virus is not transmitted within Kuwait. It may be explained by (i) the absence of *Aedes aegypti*, the classical vector of dengue<sup>(11)</sup>, and (ii) *Aedes caspius* may not be a vector<sup>(12)</sup>. However, a recent surveillance study in Saudi Arabia had revealed that in the Jeddah

area, there were 665 confirmed cases of dengue infection<sup>(13)</sup>. This was explained by the expansion of the city, which had resulted in a tremendous increase in the number of fresh water containers being used there. These proved to be the breeding sites for *Aedes aegypti*<sup>(13)</sup>. Since there is continuous expansion of residential areas in Kuwait city, the risk of the introduction of the dengue virus into the country is real. Therefore, there is no scope for complacency. *Aedes aegypti* is well entrenched in Saudi Arabia and Oman in the region. Local health authorities need to be vigilant against the introduction of this species into the country to prevent dengue endemicity.

## Acknowledgment

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# Identification of Genetic Variation among Dengue Virus DEN-4 Isolates with Heteroduplex Analysis<sup>+</sup>

by

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## Abstract

Heteroduplex Analysis (HA) was applied for the identification of genetic variation among five DEN-4 isolates. A 398 bp fragment from NS2a-NS2b region was amplified by reverse transcription/polymerase chain reaction (RT/PCR), and the products were first analysed by HA with different strain as reference strain. In order to confirm the results from HA, each RT/PCR product was also cloned into appropriate vector and were sequenced. HA results showed that the isolates from the DF epidemic in 1990 in southern China shared the same band pattern. The band pattern of the isolate from the DF epidemic in 1978 in southern China was obviously different from the band patterns produced by the 1990 isolates. Sequence results confirmed that the 1990 isolates shared the same sequence, and the sequence of the 1978 isolate was indeed different from the sequence of the 1990 isolates. These results indicated that HA can rapidly identify variations among the dengue viruses, and thus HA could be used as a useful tool in the molecular epidemiological studies of dengue virus.

**Keywords:** Dengue virus, variation analysis, Heteroduplex Analysis (HA).

## Introduction

Dengue virus is a member of *flavivirus*; it is a single-strand positive sense RNA virus; and it has four distinct serotypes, DEN-1, DEN-2, DEN-3, and DEN-4. Its genome is about 11,000 nucleotides in length. The genome

consists of a single open reading frame which encodes a precursor polyprotein. Proteolytic cleavage of the polyprotein results in the formation of the core(C), membrane (M) and envelope (E) proteins, and the nonstructural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5.

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Dengue viruses are transmitted to humans by the mosquito vectors, like *Aedes aegypti* and *Aedes albopictus*. Approximately 2.5 billion people live in areas at risk for the epidemic transmission of dengue. It can cause dengue fever (DF) and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). There are about 100 million cases of DF and 250,000 cases of DHF/DSS occurring annually. In 1978, dengue fever occurred in an epidemic form in southern China after an absence of nearly 40 years. Since then, DF has become a serious health problem in the area. The DF epidemic occurs every 2-3 years in China and the epidemic areas are enlarging, though the endemics are mainly restricted to southern China. However, molecular epidemiological studies of dengue virus in mainland China are limited<sup>(1)</sup>. Therefore, further study of dengue virus' isolates in mainland China is needed to identify genetic characteristics that may influence their epidemiology, virulence and often biological characteristics of the viruses.

Molecular techniques that have been used for the identification of genetic variation among dengue virus strains include oligonucleotides fingerprinting (ONF)<sup>(2)</sup>, primer-extension<sup>(3)</sup>, antigen signature analysis<sup>(4)</sup>, Restriction Fragment Length Polymorphisms (RFLP)<sup>(5)</sup>, Single-Strand Conformation Polymorphism (SSCP)<sup>(6)</sup>, and sequencing<sup>(7)</sup>. Nucleotide sequencing is considered to be the gold standard for the analysis of genetic variation, but because of the high expenses, it is not cost-effective for the study of large numbers of isolates as molecular epidemiological studies generally require analysis of a large number of isolates. Thus, it is difficult to use this technique to

identify and study genetic variation on a large scale.

Heteroduplex Analysis (HA) has been recently used to identify genetic variation in HIV-1<sup>(8)</sup>, influenza virus<sup>(9)</sup>, varicella-zoster virus<sup>(10)</sup>, measles virus<sup>(11)</sup>, and Norwalk-like virus<sup>(12)</sup>. HA has proved to be a sensitive method for the detection of genetic variation in viruses. HA is based on the electrophoretic mobility to its corresponding homoduplex with no mismatch<sup>(13)</sup>. This method was originally developed to detect single-base substitution in PCR products. The present study attempts the use of HA to analyse some DEN-4 isolates collected in southern China. The isolates were first amplified by RT/PCR and detected by HA. Finally, the results were confirmed by sequencing each of the strains.

## Materials and methods

Virus isolates and cell line four DV4 Chinese isolates from the 1990 epidemic in Guangdong and one DV4 Chinese isolate from the 1978 epidemic in Guangdong were chosen for analysis (Table). These isolates were from sera of DF patients using routine methods in C6/36 cell line. Their serotype were determined by Indirect Immuno Fluorescence Assay (IFA). The three isolates from the 1990 epidemic were from DF patients in the early, peak and later part of the epidemic. The GDA63 strain was isolated from *Aedes albopictus* in the early stage of the 1990 epidemic. GD7856B2 was isolated from a DF patient in the 1987 epidemic. DV4 H-241 is the prototype of DV4, which was isolated from a DF patient in the Philippines in 1956.



**Table:** Description of DV4 virus isolates compared by sequence analysis

Strains	Receiving date	Geographical origin	Source	GeneBank Accession No.
GDA63	9/15, 1990	China	<i>Aedes albopictus</i>	Y19171
GD9006A1	9/13, 1990	China	DF patient	Y19172
GD9033A1	9/30, 1990	China	DF patient	Y19173
GD9049A2	10/23, 1990	China	DF patient	Y19174
GD7856B2	1978	China	DF patient	Y19175
DV4 H-241	1956	Philippines	DF patient	Y19176

### **RNA extraction and RT/PCR amplification of viral RNA**

Viral RNA was extracted directly from virus stock as the method described by Yao HJ et al.<sup>(1)</sup>. The primers used in RT and PCR are as follows. Upstream primer: D4S, 5'-CCATTATGGCTGTGTTGTTT-3', 3973nt---3992nt; downstream primer: D4C, 5'-TTCATCCTGCTTCACTTCT-3', 4370nt---4352nt. RT was performed in 20µl of Tris-HCl 50mM, pH8.3, KCl 50mM, MgCl<sub>2</sub> 8mM, dithiothreitol 10mM, 0.5 mM dATP, 0.5 mM dCTP, 0.5mM dGTP, 0.5 mM dTTP, 40 units RNase inhibitor and two units of AMV reverse transcriptase (Promega), 100 ng down stream primer, and 100ng RNA. The reaction was incubated at 42°C for one hour and then at 99°C for 5 minutes to inactivate the AMV reverse transcriptase and degrade the RNA template. Stored at -20°C for further use.

The PCR amplification was done in 50µl reaction volume; it contained 10 mM Tris-HCl, pH 8.4, 50mM KCl, 2 mM MgCl<sub>2</sub>, 10µg gelatin, 0.25mM each dNTP (dATP, dCTP, dGTP, and dTTP), 0.3µg of upstream and downstream primers, 5µl RT reaction

product, preheated at 94°C for 5 min and then added 2.5 units of Pfu DNA Polymerase (Gibco). Amplification was done with the following parameters: denaturation at 94°C 30 s, annealing at 55°C 45 s, and extension at 72°C 45 s, 30 cycles. After the last cycle, samples were maintained at 72°C for five minutes. Take 10 µl of the reaction mixture for electrophoresis in 1% Agarose gel in 1 X TAE containing ethidium bromide (0.5µg/ml).

### **Cloning of the PCR product**

Specific PCR products were purified with GeneClean II kit (HB101, Inc). Then, this product was inserted into PCR 4Blunt-TOPO vector (Invitrogen) according to the protocols in the kit. Briefly, the product was ligated with the pCR 4Blunt-TOPO vector at appropriate ratio according to kit's manual. Take 2 µl ligation mixture for transformation. It was transformed into DH5α electro-competent cell with electroporation method (25 µF, 2.5 kV, 200 Ω). Then, plated onto LB plate containing Ampicillin (100 µg/ml).

### Identification of correct clones

Colonies on plates were picked and identified with enzyme digestion (with Eco. RI) and PCR. Heteroduplex Analysis (HA) Mix 5µl specific PCR product with an equal amount of reference isolate's PCR product, mixed well, denatured at 95°C for five minutes, then renatured at 55°C for 15 minutes. For polyacrylamide gel electrophoresis (PAGE), the renatured samples were loaded along with 2µl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol) into a 1.5mm thick, 20cm x 20cm square, 6% nondenaturing polyacrylamide gels containing 1M urea and 1% glycerol in 1 x TBE, 160V for 3 hours. After electrophoresis, the gels were stained with 0.5µg/ml of ethidium bromide in 1xTBE for 40 minutes at 4°C. DNA bands were observed under ultraviolet light.

### DNA sequencing

The nucleic acid sequencing was performed in automatic ABI PRISM 377 DNA sequencer with Bigdye Terminator Cycle Sequencing Ready Reaction Kit (PERKIN ELMER). Protocols used were depicted in the kit's manual.

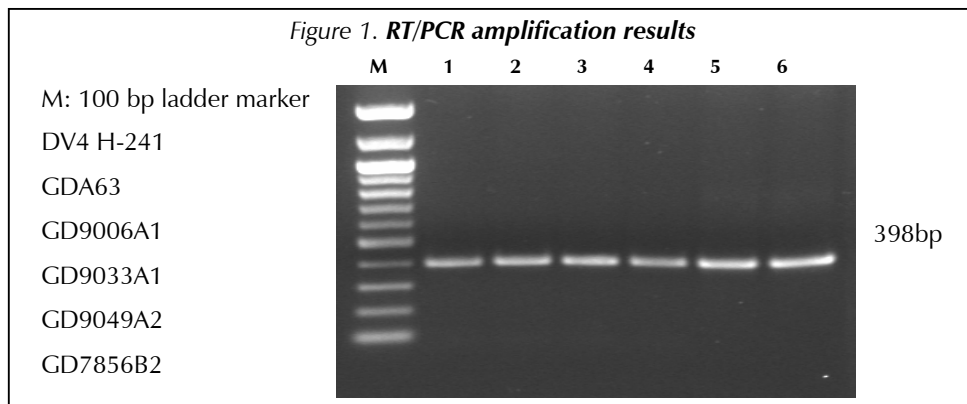
### DNA sequence analysis

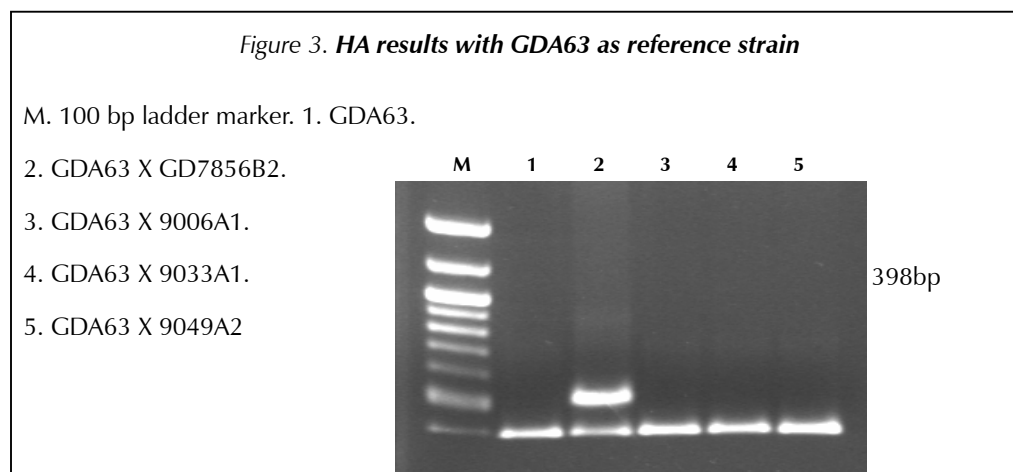
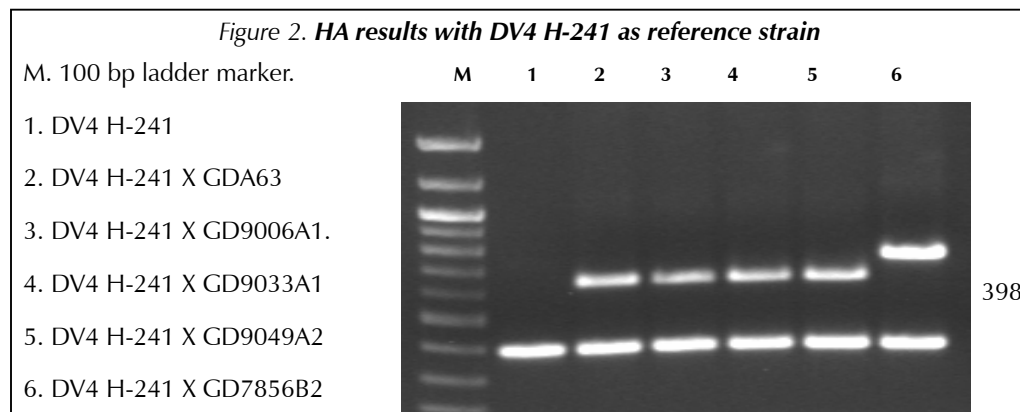
Clustal V programmes (from Baylor College of Medicine) were used to align the above sequences.

### Results

Amplification by RT/PCR A 398 bp PCR product can be obtained in all chosen isolates, as shown in Figure 1.

Analysis of the cDNA by HA. First we compared the local isolates with DV4 prototype (DV4 H-241), as shown in Figure 2. The isolates from the 1990 epidemic shared the same band pattern; the band pattern produced by GD7856B2 was obviously different from that of the isolates from the 1990 epidemic. To further identify the differences between the 1990 isolates and the 1978 isolate, HA analyses of these isolates were done further with GDA63 as reference strain, as shown in Figure 3. Because the band patterns among the isolates from 1990 were the same, they shared the same sequence. However, the band pattern produced by GD7856B2 was different from the patterns produced by the isolates from the 1990 epidemic.





HA has been used recently to identify the genetic variation in some RNA viruses like GBV, HIV-1, Influenza virus, etc. It is an effective tool in the molecular epidemiological studies of these viruses. HA is very rapid, simple to perform, and sensitive. Its size limit is up to 800 bp as compared with SSCP<sup>(14)</sup>. In this study, we tested the potential of HA to identify genetic variation among DEN-4 isolates collected at different times in southern China.

The HA band patterns of the NS2a-NS2b cDNA from the four isolates of the 1990 epidemic showed the same shift pattern; however, the pattern produced by isolate GD7856B2 from the 1978 epidemic was different (Figure 2), and the band patterns obtained with HA were highly reproducible (data not shown). To further check the difference among the isolates from the 1978 and 1990 epidemics, we used GDA63 as the reference strain (Figure 3). The band pattern showed that the

GD7856B2 sequence seemed different from the sequences of the strains from 1990.

The sequence analysis of these strains showed that there were indeed many nucleotides substitutions between the strains from the 1978 and 1990 epidemics. Sequence results also confirmed that the four isolates from 1990 shared the same sequence, and this explained why they had the same band pattern (Figures 2, 3) in HA. Another finding was that the will be seriously retarded if there are more mismatches between the heteroduplex (see Figure 2, 3).

Because GD9006A1, GD9033A1, GD9049A2 and GDA63 shared the same sequence (at least in the sequenced region), this revealed that the epidemic in southern China in 1990 was caused by the same DV4 strain. GDA63 was a strain isolated from *Aedes albopictus* in the epidemic area. Through sequence comparison, we found that it shared the same sequence with the three strains from the patients, which could be an indication of the fact that the virus was transmitted to humans from mosquitoes. Although we did not sequence the whole sequence of the four strains from 1990, it seemed that the dengue viruses were stable in this epidemic. Singh UB et al.<sup>(15)</sup> also sequenced 455 bp E-NS1 region in nine samples of DEN-2 virus isolated in India in 1996, and he found that there were some mutations among these 9 isolates in this region, but were mainly in the third position of a codon. Epidemic strains are relatively stable in one epidemic.

In dengue virus variation studies, the latest method is SSCP<sup>(1,6)</sup>. However, SSCP size limit is about 400 bp; for longer

fragment, it will fail to detect the mutations<sup>(16)</sup>. What is more, the detection rate for G $\leftrightarrow$ C transition is also lower. Conditions for SSCP must be empirically determined because the conformations of the single strand cannot be predicted. Compared with SSCP, HA has a higher size limit (up to 800bp), and it is reproducible. Unlike SSCP, HA does not rely on the secondary structure formation of ssDNA. It relies on heteroduplex formation, thus, the sample's treatment (denature and renature) is simpler than SSCP. One need not worry about quick renaturing of the ssDNA, which sometimes occurs in SSCP.

HA was proven to be a rapid and reliable tool to screen dengue virus isolates and could be used to identify which isolates should be sequenced for further molecular epidemiological studies. The results in this paper showed that HA was a helpful technique for rapidly identifying genetic variation among dengue viruses. Unlike ONF, primer-extension, antigen signature analysis, RFLP, SSCP and sequencing, HA is easy to perform, and the results can be obtained within one day. It does not need advanced equipment and expensive reagents either. Because HA cannot tell the location and number of nucleotide substitution, it cannot be used for phylogenetic studies and cannot replace sequencing. The major advantage of HA is that it can process a large number of samples in one day, and can also differentiate between the sample and the reference strain. Interesting samples selected by HA can be further studied. HA could be used as a screening tool for dengue virus in molecular epidemiological studies.

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## Rapid Detection of Dengue Viral RNA by Nucleic Acid Sequence-Based Amplification (NASBA)

by

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### Abstract

The suitability of RNA amplification by nucleic acid sequence-based amplification (NASBA) for the detection of dengue viral RNA was investigated. A set of primers and probe were synthesized, based on a selected RNA sequence from the non-coding region at the 3' end of dengue viral RNA, and was used in the NASBA assay. The NASBA reaction product was then determined by agarose gel electrophoresis and electrochemiluminescence (ECL) signal count. The sensitivity of the NASBA assay was equal to 1 PFU/ml for all of four dengue virus serotypes. There was no false positive result with Japanese encephalitis (JE) virus. This method was used successfully to detect dengue virus in the infected tissue culture cells. This test will be useful for the detection of dengue viruses in the clinical specimens.

**Keywords:** Dengue virus, Nucleic Acid Sequence-Based Amplification (NASBA), RNA, detection, electrochemiluminescence (ECL).

### Introduction

Dengue virus infection, a mosquito-borne disease, is a major cause of morbidity and mortality worldwide, especially in tropical and subtropical regions<sup>(1)</sup>. Dengue virus infection is caused by dengue virus (Family *Flaviviridae*, Genus *Flavivirus*), which has four

antigenetically distinct serotypes (DEN-1 to DEN-4). Infection with any of the dengue viruses generally leads to different severity in the patients from mild febrile, dengue fever (DF) to dengue haemorrhagic fever (DHF) or complicated with shock, dengue shock syndrome (DSS)<sup>(2)</sup>. The most challenging

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problem associated with patient management is rapid diagnosis in dengue cases.

Currently, the laboratory diagnosis of dengue virus infection is based on virus isolation and anti-dengue virus antibodies detection<sup>(3)</sup>. Isolation of dengue virus from patients' sera collected in the acute phase of illness, or from mosquito vectors, can be accomplished with cell culture or mosquito inoculation, when the virus can be detected by using specific monoclonal antibodies<sup>(4)</sup>. These methods are sensitive but time-consuming with incubation periods ranging from 5 to 14 days. Serological diagnosis, such as haemagglutination inhibition test (HI), complement fixation (CF), neutralization test, and enzyme-linked immunosorbent assay (ELISA), are commonly used in most laboratories for the detection of antibodies. These methods are simple to perform but generally require paired serum samples for the measurement of four-fold or greater rising of antibody titers and the cross-reaction of the antibodies to another flavivirus may occur<sup>(5)</sup>.

Polymerase chain reaction (PCR) is a molecular technique that has facilitated the detection of several kinds of microorganisms including dengue virus<sup>(6)</sup>. PCR is performed rapidly, and is sufficiently sensitive for the detection of all four dengue virus serotypes in any kind of specimen; however, it generally requires specialized training and specific equipment as thermalcycler.

Recently, an alternative method to PCR, the nucleic acid sequence-based amplification (NASBA) has been developed. NASBA is an isothermal RNA amplification technique that is achieved by the action of avian myeloblastosis-reverse transcriptase

(AMV-RT), T7-RNA polymerase and RNase-H<sup>(7)</sup>. In contrast to PCR, NASBA is performed without the use of specific equipment such as a thermalcycler. The amplification products can be detected by electrochemiluminescence (ECL) or agarose gel electrophoresis (AG) or enzyme-linked gel assay (ELGA). NASBA has already been successfully applied for the detection of viral RNA<sup>(8,9,10,11)</sup> and other micro-organisms<sup>(12,13)</sup>.

In this study, attempts were made to develop NASBA as a new detection method for dengue virus, and to compare the procedures for detecting NASBA products by using ECL and AG.

## Materials and methods

### *Virus strains*

Virus seeds in C6/36 cell lines were obtained from the Department of Virology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok. DEN-1 (Hawaii), DEN-2 (New Guinea C), DEN-3 (H-87) and DEN-4 (H-241) and Japanese encephalitis (JE) virus were titrated in VERO cells by a standard plaque assay.

### *Selection of primers and probes of NASBA*

The primers and probes used in this study are listed in Table 1. A pair of oligonucleotide primers is carrying T7-RNA polymerase as the specific tail that recognized the sequence at 5' end of the target RNA. NASBA products derived from this pair of primers were calculated to be 203 nucleotides, including the primer sites.

**Table 1:** Primers and probe for detection of dengue virus

	Sequence (5'to3')	Position	Size*
P1	aat tct aat acg act cac tat agg gga gac (T7 promotor) AGC AGG ATC TCT GGT CT	10638-10654	203 bp
P2	gat gca agg tcg cat atg agg gtt aga gga (ECL tail) GAC CCC TCC C	10511-10520	203 bp
Probe	AAA CAG CAT ATT GAC GCT GGG	10615-10638	154 bp

\* Use DEN-2 (New guinea C) as the reference sequence; including primer sites

### **Nucleic acid isolation**

Nucleic acid release and isolation were performed as described by Boom et al., 1990(14). Briefly, 100 µl of serum sample was added to lysis buffer solution (consisting of 4.7 M GUSCN/ 46 mM Tris-HCL, pH 6.4/ 20 mM EDTA/ 1.2% (W/V) TritonX-100). Activated silica suspension (50 µl; 1mg/ml in 0.1 M HCL) was added. The silica pellet was washed twice with washing buffer (5.25 M GUSCN/ 50 mM Tris-HCL, pH 6.4), twice with 70% ethanol and once with acetone. The pellet was dried at 56°C for 10 min. Finally, nucleic acids were eluted with elution buffer (1.0 mM Tris-HCL, pH 8.5) and stored at -20°C.

### **Nucleic acid amplification**

The reaction was performed as per the manufacture's instructions (Organon Teknika, BV, Boxtel, the Netherlands). The reaction was performed with 20 µl of reaction mixture (consisting of 40 mM Tris-HCL, pH 8.5/12 M MgCl<sub>2</sub>/70 mM KCl/1.5% (v/v) of dimethyl sulfoxide/5 mM dithiotreitol/1mM of each dNTPs/2 mM of

each ATP, CTP, UTP/1.5 Mm GTP/0.5 mM ITP/0.1 µg/µl of BSA/0.08 U RNase-H/32 U T7-RNA polymerase and 6.4 U AMV-RT/ 0.2 µM of each primer and 5 µl of isolation nucleic acid). Amplification products were stored at -20°C for further analysis.

### **Nucleic acid detection**

The reaction was carried out according to the manufacture's instructions (Organon Teknika, BV, Boxtel, the Netherlands). The amplification products were diluted to 1:20 in detection diluent (1.0 mM Tris-HCL, pH 8.5/0.2g/l methylisothiazolone), incubated with biotinylated dengue virus-specific probe bound to 5 µg of streptavidin-coated paramagnetic beads and 3 x 10<sup>11</sup> molecules of ruthenium-labelled oligonucleotides detection probe. Then 300 µl of assay buffer (100 mM tripropylamine, pH 7.5) was added before reading by an ECL reader (NASBA QR System, Model 2000; Organon Teknika, B.V., Boxtel, the Netherlands).

### **Agarose gel electrophoresis**

NASBA amplification products were analysed by agarose gel electrophoresis and visualized with ethidium bromide staining.

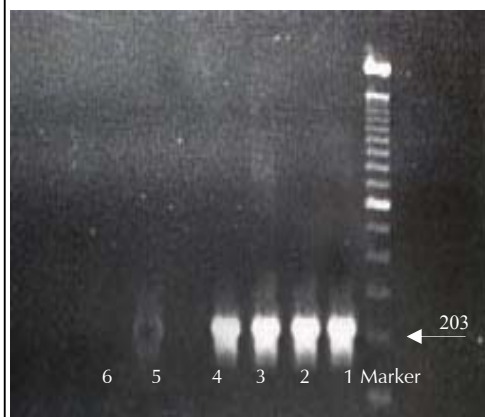


## Results

### Sensitivity of NASBA

All dengue virus serotypes were amplified with P1 and P2 and gave a clear band of 203 bp by agarose gel electrophoresis (Figure 1).

**Figure 1: Agarose gel electrophoresis of NASBA amplification products for detection of dengue viruses**



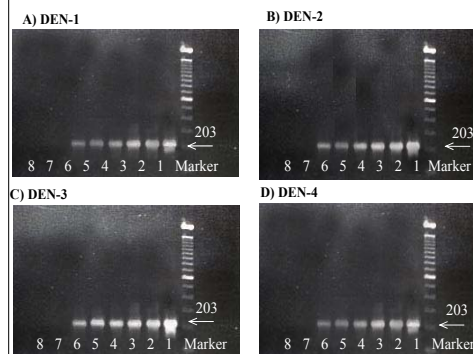
[Lane 1: DEN-1, lane 2: DEN-2, lane 3: DEN-3, lane 4: DEN-4, lane 5: negative control, lane 6: Japanese encephalitis virus. Molecular weight markers are shown on the right; DNA sizes are given in base pairs]

The lower limit of detection of each dengue virus serotype by NASBA is shown in Figure 2. The detection limit for all dengue virus serotypes after NASBA reaction was confirmed as less than or equal to 1 PFU/ml.

Amplified products of dengue viruses were tested by ECL signal count and all gave positive results. No false positive result was seen when water was used as negative control. The detection limit for all dengue virus serotypes after NASBA reaction was confirmed as less than or equal to 1 PFU/ml

when amplified products were determined by ECL signal count (Figure 3).

**Figure 2: Assessment of the detection limit for dengue viral RNA by agarose gel electrophoresis**



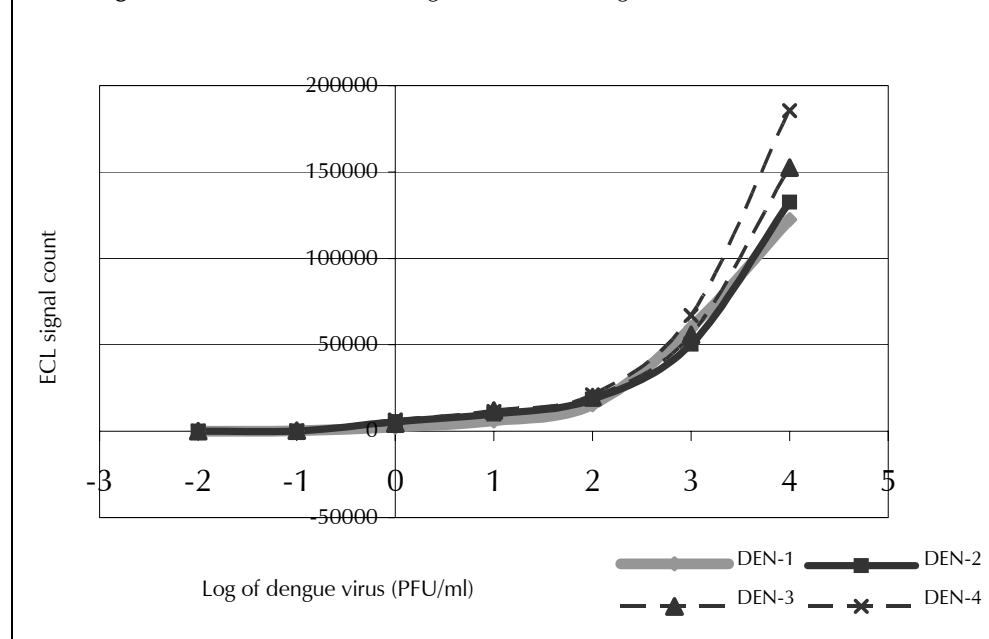
[The 10-fold dilution series of each dengue virus serotype was prepared, prior to extraction. Lane 1: 10,000 PFU/ml, lane 2: 1,000 PFU/ml, lane 3: 100 PFU/ml, lane 4: 10 PFU/ml, lane 5: 1 PFU/ml, lane 6: 0.1 PFU/ml, lane 7: 0.01 PFU/ml, and lane 8: negative control. Molecular weight markers are shown on the right; DNA sizes are given in base pairs]

### Specificity of NASBA

JE virus was extracted, amplified and detected in the same way as performed in the dengue-NASBA method described above. There was no NASBA reaction product as determined by agarose gel electrophoresis or ECL signal count. These results showed that the dengue NASBA method did not cross-react with the JE virus.

## Discussion

In this study, we developed NASBA for the detection of dengue viral RNA. The results showed that dengue viral RNA can be extracted, amplified and detected directly from virus culture. One advantage of NASBA

**Figure 3:** Detection limit of dengue viral RNA using NASBA with ECL detection

is a continuous, isothermal process that does not require a thermocycler. The constant temperature allows each step of the reaction to proceed as soon as an amplification intermediate becomes available. Thus, the exponential kinetics of the NASBA process, which are caused by multiple transcription of RNA copies from given DNA products, are intrinsically more efficient than DNA amplification methods which are limited to binary increases per cycle. It is an important research tool, particularly in the area of RNA, simplifying both RNA detection and direct sequencing. Products of NASBA are single stranded RNA, and thus can be applied to detection formats using probe hybridization without the denaturation step and contamination with genomic DNA is not amplified. The complete procedure can be undertaken in a single working day (allowing 2 hours for extraction and amplification, 1

hour for detection). In the present study, we were developing the NASBA reaction for the detection of dengue virus in clinical specimens.

## Acknowledgement

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# Detection of Dengue Viral RNA in Patients' Sera by Nucleic Acid Sequence-Based Amplification (NASBA) and Polymerase Chain Reaction (PCR)

by

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## Abstract

Nucleic acid sequence-based amplification (NASBA) was employed with a set of universal primers and probe based on the 3' non-coding region of the dengue viral RNA sequence. NASBA was used for the detection of the viral RNA in sera of patients clinically diagnosed as having dengue virus infection, and compared with polymerase chain reaction (PCR). Thirty-four acute sera were obtained from patients suspected of having dengue virus infection and 20 normal sera were obtained from primary-school children. There were 27 (50%) samples that gave a positive result for PCR, and 28 (51.85%) samples for NASBA, while 27 (50%) samples gave a negative result for PCR and 26 (48.15%) samples for NASBA. There was only one (1.85%) sample that gave a false positive result with NASBA and no false negative result was found in this study. NASBA gave 100% sensitivity, 96.30% specificity and 98.15% efficacy, respectively. NASBA will be useful in the early detection of acute dengue virus infection.

**Keywords:** Dengue virus, Nucleic Acid Sequence-Based Amplification (NASBA), Polymerase Chain Reaction (PCR), detection.

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## Introduction

Routine laboratory diagnosis of dengue virus infection often involves the detection of anti-dengue virus antibodies by haemagglutination inhibition (HI), neutralization or ELISA. These methods are simple to perform, but generally require paired sera samples for the measurement of the rising antibody titers and have high cross-reaction with other flaviviruses<sup>(1,2)</sup>. The conventional method of determining the viruses is virus isolation in tissue culture or mosquito, followed by immunofluorescent staining or ELISA typing, using specific monoclonal antibodies. The incubation period is 5 to 14 days and the technique requires good specimen handling<sup>(1)</sup>.

Polymerase chain reaction (PCR) has been widely used for the detection of many microorganisms, including dengue viruses. The assays reported by other laboratories have been concerned with different genomic regions by using different pairs of primers and different approaches for the detection of the amplification products<sup>(3,4,5,6,7)</sup>. PCR has the potential, having high sensitivity and specificity, for the detection of dengue virus in any kind of specimen. However, it generally requires a thermal cycler, which is expensive, and takes more than 12 hours for detection<sup>(3)</sup>.

Nucleic acid sequence-based amplification (NASBA) is an isothermal RNA amplification technique (usually at 41°C). NASBA reaction involves the action of 3 enzymes: avian myeloblastosis virus-reverse transcriptase (AMV-RT), RNase-H and T7-RNA polymerase; resulting in 10<sup>12</sup>fold amplification within 90 minutes<sup>(8)</sup>. The

NASBA amplification products can be detected by electrochemiluminescence (ECL) signal count, agarose gel electrophoresis (AG) and enzyme-linked gel assay (ELGA)<sup>(9,10,11,12,13,14)</sup>.

In this study, we have used NASBA and PCR to detect dengue viral RNA in the patients' sera and compared the process for determining the coupling of NASBA products by ECL and AG detection.

## Materials and methods

### *Clinical specimens*

Fourteen serum samples were obtained from inpatients clinically suspected of having the dengue virus infection and who gave a positive result with ELISA<sup>(15)</sup> from Nakhon-Phanom Hospital during the period June to October 1999. Twenty serum samples of negative controls were collected from primary school students who resided in Nakhon Phanom province. Another 20 sera were obtained from the Department of Virology, Armed Forces Research Institute of Medical Science (AFRIMS).

### *RNA extraction*

Viral RNA was isolated by using phenol-guanidine-isothiocyanate as described by Lanciotti *et al.*, 1992<sup>(4)</sup>. Viral RNA was precipitated by the addition of 500 µl isopropanol, then was centrifuged at 12,000 rpm, at 20°C for 10 minutes. The RNA pellet was washed with 75% ethanol. The pellet was dried and re-suspended with 25 µl of DEPC-treated water.

### Reverse transcriptase-polymerase chain reaction

After RNA extraction, target RNA was amplified by using RT-PCR according to the method of Lanciotti *et al.*<sup>(4)</sup>. Briefly, 2.5 µl of target RNA was mixed with RT-PCR mixture (this consisted of 100 mM Tris base, pH 8.3/500 mM KCl/15 mM MgCl<sub>2</sub>/0.1% gelatin/2.5 mM dNTPs/10 pmol of each primer/0.1m DTT/1U reverse transcriptase and 5U Amplitaq); the final volume of the reaction was 50 µl. RT-PCR was conducted at 42°C for 60 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 1 minute and extension at 72°C for 2 minutes.

### Nested-polymerase chain reaction

A second amplification reaction was initiated with 5 µl of 1:100 diluted cDNA from the

RT-PCR reaction. The reaction mixture contained all the components as described for RT-PCR, with the following exceptions: primer D2 was replaced with the specific probe TS1, TS2, TS3 and TS4; and DTT and reverse transcriptase were eliminated. Amplification was set for 20 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 1 minute and extension at 72°C for 2 minutes.

### Selection of primers and probes of the NASBA method

The primers and probe used in this study are listed in Table 1. There was a pair of oligonucleotide primers carrying T7-RNA polymerase as the specific tail that recognized the sequence at the 5' end. The size of amplicon was calculated to be approximately 203 nucleotides, including primer sites.

**Table 1:** Primer and probe for detection of dengue virus

	Sequence (5'to3') NASBA	Position	Size
P1	aat tct aat acg act cac tat agg gga gac (t7 promotor) AGC AGG ATC TCT GGT CT	10638-10654	203*
P2	gat gca agg tcg cat atg agg gtt aga gga (ECL tail) GAC CCC TCC C	10511-10520	203*
Probe	AAA CAG CAT ATT GAC GCT GGG	10615-10635	154*
<b>PCR</b>			
D1	TCA ATA TGC TGA AAC GCG CGA GAA ACC	134-161	511
D2	TTG CAC CAA CAG TCA ATG TCT TCA GGT TC	616-644	511
TS1	CGT CTC AGT GAT CCG GGG G	568-586	482
TS2	CGC CAC AAG GGC CAT GAA CAG	232-252	119
TS3	TAA CAT CAT CAT GAG ACA GAG C	400-421	290
TS4	CTC TGT TGT CTT AAA CAA GAG A	506-527	392

\* Use DEN-2 (New guinea C) as the reference sequence; including primer sites

### **Nucleic acid isolation**

Nucleic acid release and isolation was performed as described by Boom *et al.*, 1990<sup>(16)</sup>. Briefly, 100 µl of serum sample was added to lysis buffer solution (consisting of 4.7 M GUSCN/46 mM Tris-HCL, pH 6.4/20 mM EDTA/1.2% (W/V) TritonX-100). 50 µl of activated silica suspension (1mg/ml in 0.1 M HCL) were added. The silica pellet that contained nucleic acid was washed twice with wash buffer (5.25 M GUSCN/50 mM Tris-HCL, pH 6.4), twice with 70% ethanol and once with acetone. The pellet was dried at 56°C for 10 minutes. Finally, nucleic acids were eluted with elution buffer (1.0 mM Tris-HCL, pH 8.5) and stored at -70°C.

### **Nucleic acid amplification**

The reaction was performed as described by the manufacturer's instructions (Organon Teknika, B.V., Boxtel, the Netherlands). The reaction was performed with 20 µl of reaction mixture (consisting of 40 mM Tris-HCL, pH 8.5/12 M MgCl<sub>2</sub>/70 mM KCl/1.5% (v/v) of dimethyl sulfoxide/5 mM dithiotreitol/1mM of each dNTPs/2 mM of each ATP, CTP, UTP/1.5 Mm GTP/0.5 mM ITP/0.1 µg/µl of BSA/0.08 U RNase-H/32 U T7-RNA polymerase and 6.4 U AMV-RT/0.2 µM of each primer and 5 µl of isolation nucleic acid). The NASBA reaction mixture was incubated at 65°C for 5 minutes, before the enzyme solution was added to allow for destabilization of secondary RNA structures. Then it was immediately cooled down to 41°C for 5 minutes, to allow primer annealing. After the enzyme solution was added, reaction mixtures were incubated at 41°C for at least 90 minutes. The amplification product was stored at -70°C for further analysis.

### **Nucleic acid detection**

The reaction was carried out according to the manufacturer's instructions (Organon Teknika, BV, Boxtel, the Netherlands). The amplification products were diluted to 1:20 in detection diluent (1.0 mM Tris-HCL, pH 8.5/0.2g/l methylisothiazolone) and were incubated at 41°C for 30 minutes with a biotinylated dengue virus specific probe bound to 5 µg of streptavidin coated paramagnetic beads and 3x10<sup>11</sup> molecules of ruthenium-labeled oligonucleotide detection probe. During the incubation period, the hybridization mixtures were agitated every 10 minutes to keep the beads in suspension. Finally, 300 µl of assay buffer (100 mM tripropylamine, pH 7.5) were added and the test was performed in an ECL reader (NASBA QR system, Model 2000; Organon Teknika, BV, Boxtel, the Netherlands) for final reading of the results.

### **Agarose gel electrophoresis**

PCR and NASBA amplification products were analysed by agarose gel electrophoresis and visualized with ethidium bromide staining. The mixture of amplicon and gel loading buffer (50% glycerol/0.1M EDTA, pH 8.0/1% SDS/0.1% bromphenol blue/0.0% xylene cyanol) were loaded in 1.5% agarose in 1x TBE (89 mM tris/89 mM boric acid/2 mM EDTA, pH 8.0). A 100-bp ladder was used as a size standard (Gibco, BRL).

## **Results**

### **Sensitivity of NASBA**

A total of 54 sera were examined using PCR and NASBA methods. These samples included 14 acute sera with ELISA positive,

20 normal sera with ELISA negative and an additional 20 from the Department of Virology, AFRIMS. For the PCR method, 27 (50%) patients gave positive results and another 27 (50%) patients gave negative results. The results of sera showed that 28 (51.85%) patients had positive results and 26 (48.15%) patients had negative results with the NASBA method that detected the amplicon both by ECL signal count and agarose gel electrophoresis (Table 2).

**Table 2:** Comparison of dengue virus infection determination by nucleic acid sequence-based amplification and polymerase chain reaction in clinical and normal specimens

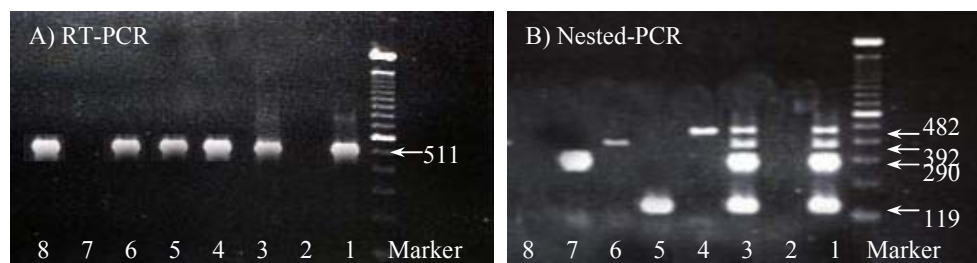
Method	Positive		Negative	
	No.	%	No.	%
PCR	27	50.0	27	50.0
NASBA with AG detection	28	51.85	26	48.15
NASBA with ECL detection	28	51.85	26	48.15

Case = 54 Cochran's Q test = 2.00 df = 2 P = 0.368

The different types of dengue viruses, both of PCR and NASBA methods that were determined by agarose gel electrophoresis, are shown in Figure 1 and Figure 2. For the PCR method, the size of the RT-PCR amplification products was 511 base pairs, and the size of the nested-PCR amplification products were 482 base pairs for DEN-1, 119 base pairs for DEN-2, 290 base pairs for DEN-3 and 392 base pairs for DEN-4. For the NASBA method, the DNA amplification product's size was 203 base pairs, including primer sites, as shown in Figure 2.

The differences of NASBA with agarose gel electrophoresis (AG) detection and NASBA with electrochemiluminescence (ECL) detection, when compared with the PCR method, were not statistically significant ( $P = 0.368$ ), as shown in Table 2. When compared with the PCR method, the sensitivity of NASBA with AG detection and NASBA with ECL detection were 100% for both; the specificity of NASBA with AG detection and NASBA with ECL detection were 96.30% for both. The efficacy, positive predictive value and negative predictive value of both detection methods were 98.15%, 96.43% and 100%, respectively.

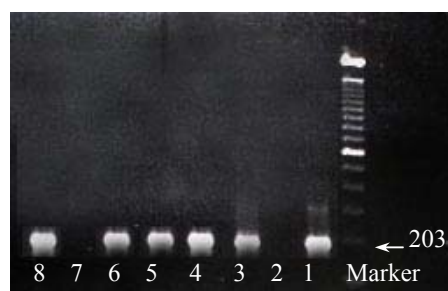
**Figure 1:** Agarose gel electrophoresis of the dengue viral RNA in the patients' sera and normal healthy controls' sera amplified by PCR method



Lane 1: positive control, lane 2: negative control, lane 3: dengue virus types 1-4, lane 4: dengue virus type 1, lane 5: dengue virus type 2, lane 6: dengue virus type 3, lane 7: dengue virus type 4, lane 8: negative control. Molecular weight markers are shown on the right RNA sizes are given in base pairs



Figure 2: Agarose gel electrophoresis of dengue viral RNA amplified by NASBA method



Lane 1: positive control, lane 2: negative control, lane 3: dengue virus types 1-4, lane 4: dengue virus type 1, lane 5: dengue virus type 2, lane 6: dengue virus type 3, lane 7: dengue virus type 4, lane 8: negative control. Molecular weight marks are shown on the right RNA sizes are given in base pairs

## Discussion

For RNA detection, several laboratories used NASBA for the detection of viral RNA<sup>(9,11)</sup>. In this study, we used Lanciotti's method to perform dengue viral RNA detection. The number of reported cases of dengue virus infection in Thailand, including Nakhon Phanom province, came down in 1999 after the campaign launched by the dengue control programme (unpublished data from Dengue Haemorrhagic Fever Control Office, 1999). Therefore, it was necessary to obtain another 20 sera from the Department of Virology, AFRIMS, to perform the test and calculate the statistics.

The obvious disadvantage of the PCR method is the need for a thermal cycler for the change in temperature during the amplification period. This instrument is relatively expensive, so it may not be appropriate in rural hospitals where budgets are limited. The second disadvantage is that the method is time-consuming; it takes at least 1.5 hours for RNA extraction, 3.5 hours for RT-PCR, 2 hours for nested-PCR and 3 hours for agarose gel electrophoresis<sup>(4)</sup>. The

other disadvantage is the need for a lot of reagents and enzymes, such as dNTPs reverse transcriptase, etc. These reagents are used at varying concentrations and volumes for each method<sup>(4,5,6,7)</sup>.

For dengue virus detection, sample collection should be done early in the viremia phase. Viral load is related to both the height of the viremia and its duration<sup>(17)</sup>. The duration of dengue viremia is prolonged in patients who have primary dengue virus infection as compared with those who have secondary dengue virus infection (5.1 days and 4.4 days, respectively). It should be noted that a precise measurement of the duration of viremia is not possible due to the onset of illness being unknown. Usually, children are enrolled up to 72 hours after the onset of the illness. The virus titer will decrease when the antibodies to dengue virus increase. The peak of stable virus titer is found in the first 2 days of acute sera. The virus can be detected after 5 days of defervescence in patients with secondary dengue virus infection and more than 5 days in primary dengue virus infection<sup>(3,4)</sup>.

NASBA is enzymatic amplification with the ability to amplify the target RNA at isothermal temperature (usually at 41°C) so that the thermal cycler is not necessary. To control the temperature during amplification, a water bath can be used instead. NASBA is available as a commercial kit that contains reagents and enzymes, so buffer or reagent preparation is not necessary. The time needed for processing, using the NASBA method, is 45 minutes for RNA extraction, 2 hours for amplification, 45 minutes for ECL detection and 3 hours for AG detection.

The cell cultures of dengue virus serotypes 1-4 and Japanese encephalitis virus were used to test the primers and probe, and also to determine the sensitivity and specificity of the test. We found that the detection limit of both NASBA with AG detection and NASBA with ECL detection was equal to 1 PFU/ml, and no cross-reaction with Japanese encephalitis virus was found in this study<sup>(18)</sup>. In terms of the specificity of the primers and the probe of the PCR method, cross-reaction can occur in the RT-PCR step (primer D1&D2) with Japanese encephalitis virus and West Nile virus, but it is different in the base pair (550 bp) from the dengue virus (511 bp). No cross-reaction is found in the nested-PCR<sup>(4)</sup>. When compared to the PCR method, the sensitivity of both NASBA with AG detection and NASBA with ECL detection was 100%; the specificity of NASBA with AG detection and NASBA with ECL detection was 96.30%. The results of NASBA with AG detection and NASBA with ECL detection were not statistically different from those of the PCR method (Cochran's Q test,  $P = 0.368$ ). The study of Wu *et al.*, 2001<sup>(10)</sup> was able to

detect dengue viral RNA at 1 to 10 PFU/ml in tissue culture and below 25 PFU/ml in clinical specimens. The sensitivity of the NASBA of Wu *et al.*, 2001<sup>(10)</sup> was 98.5% and its specificity was 100% when compared to the virus isolation method; this indicated that NASBA is a method with high sensitivity and specificity in the detection of dengue viral RNA.

There were some false negative results for NASBA methods. However, they gave positive results after repeating the test. This was due to silica dioxide in the NASBA extraction stage. The silica dioxide particle has the ability to bind with protein, nucleic acid, carbohydrate and fat, so it can bind to enzymes, extracted nucleic acid and the primers in the amplification reaction, resulting in interference with the amplification<sup>(16)</sup>.

In ECL detection, the ECL reader was limited to the NucliSens™ basic kit software version 1.0 and the reading was adjusted by performance control (PC) and cut-off level. After the PC was diluted with the normal human serum to 100-fold dilution, the PC signal was higher than the cut-off level. This was due to the PC-RNA solution containing GuSCN in a concentration that inhibited the NASBA reaction. By 100-fold dilution or greater of the PC solution, the inhibitory effect was abolished and the RNA concentration remained high enough for direct amplification (unpublished data from Help Desk of Teknika Co. Ltd.).

Both NASBA with ECL detection, and NASBA with AG detection, gave the same sensitivity and specificity. The time required for processing in the detection step was 45

minutes for NASBA with ECL detection and 3 hours for NASBA with AG detection. The disadvantage of ECL detection is the need for an ECL reader, which is specific and an expensive equipment.

NASBA with AG detection, took 4 times longer than ECL detection. The other disadvantage is toxicity from a chemical reagent, such as EDTA, ethidium bromide, and exposure to UV light, if the protection is not performed carefully. As for cost-effectiveness, NASBA with AG detection is cheaper than ECL detection.

## Conclusion

For the NASBA method, the detection limit for all four dengue virus serotypes (DEN-1 to DEN-4) was equal to 1 PFU/ml in both AG detection and ECL detection. When compared with the PCR method, both NASBA with AG detection and NASBA with ECL detection had the same sensitivity and specificity (sensitivity: 100%; specificity

96.30%; efficacy 98.15%). This difference is not statistically significant. NASBA could be performed faster than PCR but this technique is expensive and requires refinement in the laboratory for quantification and typing of the dengue viruses. Also, NASBA must be further conducted for testing the specificity of the primers and probe in other flaviviruses.

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We acknowledge the support of M/s Organon (Thailand) Ltd. for training laboratory staff and providing two diagnostic kits; the Department of Virology, Armed Forces Research Institute of Medical Science (AFRIMS), for providing the equipment and the tissue culture of four dengue viruses and the Japanese encephalitis virus; the Department of Virology, Faculty of Medicine, Ramathibodhi Hospital for providing the NucliSens Reader, and Nakhon Phanom Hospital for sample collection.

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# Intravenous Fluid Therapy in Dengue Haemorrhagic Fever – Sri Lanka Experience

by

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## Abstract

In a study carried over four months (December 2000-March 2001) 59 laboratory-confirmed cases of DHF were detected, of which 27 had grade I, 14 had grade II, 12 had grade III and 6 had grade IV DHF. In the treatment of grade I and II DHF, Hartmann's solution was given in all cases. Fresh frozen plasma (FFP) was used in 2 patients. Platelet transfusions were not given to any of the patients. The maximal rate of infusion was 3 ml/kg/hr in 9 patients, 2 ml/kg/hr in 20 patients and 1 ml/kg/hr in 12 patients. The duration of the therapy was 12-24 hours in 22 cases, 25-36 hours in 17 cases and 37-48 hours in 2 cases. In the treatment of grade III and IV DHF (DSS), Hartmann's solution was given in all cases. FFP was given to 7 patients and Dextran-70 to 1 patient. Fifteen patients were given IV boluses of Hartmann's solution/FFP/Dextran-70 (10-20 ml/kg). Three patients were not given any IV boluses. Platelet transfusions were not given to any of the patients. The maximal rate of infusion (in between boluses) was 6 ml/kg/hr in 1 patient, 5 ml/kg/hr in 12 patients, 3 ml/kg/hr in 3 patients and 2 ml/kg/hr in 2 patients. The duration of the therapy was 12-24 hours in 3 cases, 25-36 hours in 7 cases and 37-48 hours in 8 cases.

**Keywords:** Intravenous fluid therapy, dengue haemorrhagic fever, Colombo, Sri Lanka.

## Introduction

The original guidelines for the intravenous (IV) fluid therapy in dengue haemorrhagic fever (DHF) were developed at the Children's Hospital, Bangkok, by Dr Suchitra Nimannitya<sup>(1)</sup>. These guidelines have been further modified<sup>(2,3)</sup>. At the Lady Ridgeway Hospital, Colombo, for the past several years, IV fluid therapy in non-shock cases of DHF has been given at a rate much lower than the 6 ml/kg/hr recommended in the 1999 WHO

guidelines<sup>(3)</sup>. Furthermore, in dengue shock syndrome (DSS), IV boluses have been used in preference to the continuous high rates of infusion. With this form of therapy, there were only two deaths among the 354 suspected cases of DHF admitted to the Lady Ridgeway Hospital (LRH) in 1996, which was an epidemic year<sup>(4)</sup>. We thought it appropriate to carry out a prospective study of DHF to formulate guidelines for the intravenous (IV) fluid therapy based on the experience gained at LRH.

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## Materials and methods

The study was carried out in five paediatric medical units of LRH from 1 December 2000 to 31 March 2001, a period of four months. Only laboratory-confirmed cases of DHF were included in the study. A combination of thrombocytopenia (platelet count  $<150 \times 10^9/l$ ) and haemoconcentration (haematocrit  $>40\%$  or presence of lamellar pleural effusion) was considered necessary for laboratory confirmation of DHF. All patients were monitored on a two- or three-hourly basis, and even more frequently in shock cases with the assessment of vital signs such as pulse and blood pressure. Haematocrit assessments were done frequently. All patients were initially started on IV fluid therapy. The rate of IV fluid replacement was adjusted based on vital signs and haematocrit. Platelet counts were done once or twice daily. Serum transaminase levels were done at the time of admission. Whenever possible, a chest X-ray was taken in the right recumbent position. The house officers of the five paediatric medical units were responsible for data collection and maintenance of records.

- DHF was classified into four grades<sup>(5)</sup>.
- Grade I - No spontaneous bleeding
- Grade II - Spontaneous bleeding
- Grade III - Circulatory failure manifested by rapid, weak pulse and narrow pulse pressure (20 mm Hg or less)
- Grade IV - Undetectable blood pressure and pulse.

## Results

During the four-month study period there were 59 laboratory-confirmed cases of DHF. Of these, 29 were male and 30 female. Four patients were less than one year of age, 27 were 1-6 years of age, and 28 were 7-12 years of age. Twenty-seven patients had grade I DHF, 14 had grade II DHF, 12 had grade III DHF and 6 had grade IV DHF. A positive Hess's test was found in 37 patients. The serum transaminase levels were raised in 30 children. Forty-six patients had lamellar pleural effusions. While the platelet counts were lower than  $150 \times 10^9/l$  in all cases, 51 had counts less than  $100 \times 10^9/l$  and 13 had counts less than  $50 \times 10^9/l$ . Haematocrit assessments were done twice daily in 38 patients, thrice daily in 7 patients, 4 times daily in 7 patients and 5 or more times daily in 7 patients. The haematocrit exceeded 40% in 53 children. In the 6 children where the initial haematocrit was less than 40%, lamellar pleural effusions were present. In these 6 children the convalescent haematocrit was 20% less than the initial level.

### ***Treatment of grades I and II DHF (non-shock DHF)***

There were 41 patients in this category. The type of fluid used for the IV therapy consisted of Hartmann's solution with or without dextrose. In addition, fresh frozen plasma (FFP) was used in 2 patients. Platelet transfusions were not given in any of the patients. The maximal rate of infusion was 3 ml/kg/hr in 9 patients, 2 ml/kg/hr in 20 patients and 1 ml/kg/hr in 12 patients. The duration of therapy was 12-24 hours in 22 cases, 25-36 hours in 17 cases and 37-48

hours in 2 cases. In no patient was IV fluid given for longer than 48 hours. There were no complications.

### ***Treatment of grades III and IV DHF (DSS)***

There were 18 patients in this category. While Hartmann's solution with or without dextrose was given to all patients, FFP was additionally given to 7 patients and Dextran-70 to 1 patient. Fifteen patients were given one or more IV boluses of Hartmann's solution (10-20 ml/kg). Four patients were additionally given IV boluses of FFP (10 ml/kg) and 1 patient was additionally given an IV bolus of Dextran-70 (10 ml/kg). Three patients were not given any IV boluses. Platelet transfusions were not given to any of the patients. The maximal rate of infusion (in between boluses) was 6 ml/kg/hr in 1 patient, 5 ml/kg/hr in 12 patients, 3 ml/kg/hr in 3 patients and 2 ml/kg/hr in 2 patients. The duration of the therapy was 12-24 hours in 3 cases, 25-36 hours in 7 cases and 37-48 hours in 8 cases. There were no deaths and all patients recovered uneventfully.

## **Discussion**

According to the 1999 WHO guidelines<sup>(3)</sup> it is recommended that in the treatment of grades I and II DHF, IV fluid therapy be commenced with Hartmann's solution or dextrose-saline at the rate of 6 ml/kg/hr. Once improvement occurs as shown by a falling haematocrit, good volume pulse and stable blood pressure, the rate is reduced to 5 ml/kg/hr, then to 3 ml/kg/hr and discontinued after 24-48 hours. At LRH the maximal rate of infusion used in the treatment of grades I and II DHF was 3

ml/kg/hr and in 22 cases (54%) the duration of IV fluid therapy did not exceed 24 hours and in only 2 cases exceeded 36 hours. No complications were encountered using this regime.

In the treatment of DSS the 1999 WHO guidelines<sup>(3)</sup> recommend that Hartmann's solution or dextrose-saline be infused at the rate of 10-20 ml/kg/hr or, in the case of profound shock (grade IV), in the form of a bolus (10 ml/kg) of Hartmann's solution or Dextran-40. At LRH IV boluses (10-20 ml/kg) were given whenever the pulse pressure was 20 mm Hg or less in both grade III and IV cases. Continuous infusions at the rate of 10-20 ml/kg/hr were rarely given because of the greater risk of excess fluid administration. Fifteen of the 18 patients with DSS were given one or more IV boluses of Hartmann's solution, followed in a few cases by IV boluses of FFP or Dextran-70. Dextran-70 was used because Dextran-40 was not available at LRH. In 3 patients no IV boluses were used and in between boluses the maximal rate of infusion used was 6 ml/kg/hr.

Two patients with non-shock DHF and 7 patients with DSS were given FFP purely as a form of volume replacement. Disseminated intravascular coagulation (DIC) was not found in any of these cases. No doubt this is an acceptable form of treatment but it would have been preferable to have used Dextran-40 instead, thus avoiding the use of blood products. Ideally, use of FFP should be limited to situations where DHF is complicated by DIC. The cost of a 500 ml pack of Dextran-40 is only 180 Sri Lankan rupees. Comparatively, the cost of a 200-250 ml pack of FFP is 400 Sri Lankan rupees. Thus the use of Dextran-40 is cost-

effective and it is time that we ensured that Dextran-40 is freely available in our hospitals. Before infusion of Dextran-40, blood should be taken for cross-matching. The maximum volume of Dextran-40 which should be used is 30 ml/kg of body weight<sup>(3)</sup>. It is heartening to note that platelet transfusions were not given at LRH even though 13 patients (22%) had platelet counts less than  $50 \times 10^9/l$ . This is in complete accordance with the WHO guidelines<sup>(3)</sup>.

At LRH, in 64% of the children, haematocrit assessments were done only twice daily. Obviously, in the outstation hospitals the situation will be even worse. Thus, adjustments of fluid rates according to haematocrit estimations will be done only once or twice daily. By following the WHO recommendations the initial fluid rate of 6 ml/kg/hr will be reduced to 5 ml/kg/hr only at the end of 12 hours and further reduced to 3 ml/kg/hr after 24 hours with no further reduction until the drip is discontinued. Such a child is likely to have massive pleural effusion, ascites, pulmonary congestion and oedema. Due to the low fluid rates used by us, this problem did not arise in our patients. It should be stressed that there is no substitute

for frequent monitoring of patients even if regular haematocrit values are not available.

## Recommendations

1. For grades I & II DHF we recommend a maximal fluid rate of 3 ml/kg/hr with discontinuation of IV fluid therapy within 36 hours. Hartmann's solution or dextrose-saline usually suffice.
2. For grades III & IV DHF we recommend a maximal fluid rate of 5 ml/kg/hr. In addition IV boluses of Hartmann's solution/Dextran-40/FFP (10-20 ml/kg) should be given when the pulse pressure is 20 mm or less.
3. If the haematocrit is falling but the child is deteriorating, fresh whole blood transfusion (10 ml/kg) should be given without delay.
4. Platelet transfusions are indicated along with FFP when DIC causes significant bleeding.

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# Inflammatory Mediators in Dengue Virus Infection: Circulating Interleukin-12 and Interferon- $\gamma$

by

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## Abstract

Interleukin (IL)-12 stimulates the production of interferon (IFN)- $\gamma$  by T or NK cells, is a growth factor for preactivated T and NK cell, and enhances the cytotoxic activity of cytotoxic T cells and NK cells. To investigate the potential role of the IL-12/IFN- $\gamma$  axis in dengue virus infection, we measured circulating levels of the p40-subunit of IL-12 and those of IFN- $\gamma$  in 186 patients with this disease, and in 33 apparently healthy children as well as in 11 patients with bacterial infections as positive controls. Levels of IL-12-p40 were elevated in 90%, whereas those of IFN- $\gamma$  were increased in 36.4% of the patients, respectively. Apparently healthy endemic children had similar levels of IL-12-p40, whereas they had significantly lower IFN- $\gamma$  ( $p < 0.01$ , WMW-test). In contrast, the patients with bacterial infections had similar levels of IL-12-p40 and IFN- $\gamma$  as compared to the dengue patients. The levels of both cytokines were higher in the dengue patients without shock than in those with dengue shock syndrome ( $p < 0.01$ ). The IL-12-p40 and IFN- $\gamma$  levels correlated with each other ( $r = 0.2$ ,  $p = 0.00$ ; two-tailed Spearman rank correlation). In addition, the IL-12 levels correlated with plasma protein levels, haematocrit value and the presence of ascites ( $r = 0.15$ ,  $p = 0.04$ ;  $r = -0.25$ ,  $p = 0.001$ ;  $r = 0.35$ ,  $p = 0.00$ , respectively). IFN- $\gamma$  correlated with plasma protein levels, platelet counts and the presence of ascites ( $r = 0.2$ ,  $p = 0.00$ ;  $r = -0.20$ ,  $p = 0.004$ ;  $r = 0.23$ ,  $p = 0.002$ , respectively).

The results of this study indicate that there was no significant IL-12-p40 response in dengue patients as compared to controls, whereas IFN- $\gamma$  levels were increased in a substantial number of patients with dengue virus infection. The findings do not allow for firm conclusions on the role of the IL-12/IFN- $\gamma$  axis in the pathogenesis of dengue virus infection.

**Keywords:** Children, dengue virus infection, interferon-gamma, interleukin-12, shock.

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## Introduction

Dengue is an acute infectious viral disease characterized by biphasic fever, headache, pain in various parts of the body, prostration, rash, lymphadenopathy, and leukopenia<sup>(1,2)</sup>. Dengue virus infection presents itself as two major clinical syndromes, dengue fever (DF) and dengue haemorrhagic fever (DHF)<sup>(1)</sup>. DF, the most common type of dengue illness, is a self-limited febrile disease. DHF is a more serious illness sometimes complicated by dengue shock syndrome (DSS). Based on data primarily collected in Thailand, the World Health Organization has proposed definitions for DHF and DSS, and recognizes four grades of severity<sup>(3)</sup>:

- DHF Grade I: Fever accompanied by non-specific symptoms with a positive tourniquet test as the haemorrhagic manifestation;
- DHF Grade II: Grade I accompanied by spontaneous haemorrhagic manifestation;
- DHF Grade III: Circulatory failure manifested by tachycardia with narrowing of the pulse pressure (<20 mmHg) or hypotension, and
- DHF Grade IV: Profound shock with undetectable blood pressure and pulse.

At present, the pathogenesis of dengue haemorrhagic fever is incompletely understood. Some studies suggest the involvement of T-cell responses in the pathogenesis of DHF and DSS: CD4<sup>+</sup> cells from humans having suffered from dengue proliferate producing gamma-interferon (IFN- $\gamma$ ) in response to soluble dengue virus antigen<sup>(4)</sup>; circulating levels of sIL2R, sCD4

and sCD8 are significantly higher in patients with DHF than in healthy children<sup>(5)</sup>; serotype-cross-reactive C4<sup>+</sup>CD8<sup>-</sup> cytotoxic T-lymphocyte clones secreting interferon (IFN)- $\gamma$  have been isolated from patients<sup>(6,7)</sup>; peripheral blood mononuclear cells from a dengue-4 immune donor have been shown to proliferate in response to a live dengue virus<sup>(6)</sup>; and, finally, serotype-cross-reactive, CD8<sup>+</sup>, class I-restricted, dengue virus-specific cytotoxic lymphocytes have been identified and it has been suggested that these cells may mediate viral clearance and contribute to shock by lysing dengue virus-infected cells in secondary infections<sup>(6)</sup>.

Interferon-gamma (IFN- $\gamma$ ) is a main cytokine released by CD8 effector T cells, capable of blocking viral replication<sup>(8)</sup>. IFN- $\gamma$  is also a potent activator for phagocytic cells, increasing their bactericidal activity as well as their ability to produce cytokines. A major factor produced by infected phagocytes responsible for the induction of IFN- $\gamma$  synthesis is interleukin-12 (IL-12). IL-12 consists of a heterodimer and is a potent inducer of cytokine production, particularly IFN- $\gamma$ , in T and NK cells. In addition, it is a growth factor for preactivated T and NK cells, and an enhancer of cytotoxic activity of both CD8<sup>+</sup> T cells and NK cells<sup>(9,10,11,12,13)</sup>.

Considering a potential role for cytotoxic T lymphocytes in the pathogenesis of dengue, we hypothesized that the cytokines IL-12 and IFN- $\gamma$  might be important in the pathogenesis of dengue virus infection. Hence, in this study we investigated the release of these cytokines in patients with dengue viral infection: plasma levels of the IL-12 p40 subunit as well as those of IFN- $\gamma$  were measured in patients with dengue fever or dengue haemorrhagic

fever in comparison to those in healthy children or patients with bacterial infections.

## **Materials and methods**

The patients included in the study were admitted to the Department of Paediatrics of the Dr Sardjito General Hospital in Yogyakarta, Indonesia, between September 1995 and May 1996. They presented with fever lasting 2-7 days. A clinical diagnosis of DF or DHF was made according to the WHO criteria before the results of serological studies were known. The severity of illness was graded according to the WHO criteria for dengue haemorrhagic fever<sup>(3)</sup>. A definitive diagnosis of dengue virus infection was made when patients had elevated levels of IgM antibodies with or without detectable IgG antibodies against a dengue virus according to the criteria described in the interpretation of MAC-ELISA results<sup>(3)</sup>.

Two groups of patients served as controls. Patients with a bacterial infection (bacterial meningitis, sepsis or typhoid fever) served as "positive" controls. The diagnosis of these infections was based on clinical symptoms in combination with the results of cerebrospinal fluid culture and an elevated cell in the cerebrospinal fluid, blood culture, or a Widal serological test, respectively. Apparently healthy children in the outpatients department served as "negative" controls.

The clinical signs were registered in all patients at the time blood was collected for the present study. All patients were treated with supportive therapy, including infusion of crystalloid, plasma or whole blood when necessary, as a standard therapy. The protocol was approved by the Medical Ethics Committee of the Faculty of Medicine,

Gadjah Mada University, Dr Sardjito Hospital. Informed consent was obtained from the parents of each patient included in the study.

## **Blood sampling**

Blood was obtained from each patient within 1 day after admission and in a substantial number of patients also on subsequent days during hospital stay. Blood was collected in tubes containing soybean inhibitor (SBTI; Sigma Chem. CO., St. Louis, Mo), benzamidine and EDTA (100 ug per ml; 10mM and 10mM, final concentrations, respectively) to prevent activation of cells and of plasma cascade systems. The tubes were centrifuged for 10 minutes at 1.300 x g, and plasma was stored at -70°C in aliquots. The samples were transported on dry ice to Amsterdam, where they were stored at -70°C until tests were performed.

## **Laboratory investigations**

IgM and IgG antibodies against dengue virus were measured in the Laboratory for Exotic Viral Infections, Institute of Virology, Erasmus Medical Centre, Rotterdam, the Netherlands. Briefly, an ELISA was used to detect DEN-2-specific IgG antibodies as described earlier<sup>(14)</sup>. IgM antibodies against the recombinant E-protein were measured with a direct ELISA as described for the IgG antibody detection using an anti-IgM conjugate (Dakopatts, Glostrup, Denmark)<sup>(14)</sup>. IL-12-p40, IFN- $\gamma$ , IL-6, and IL-8 were measured with ELISAs obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, according to the manufacturer's instruction. Results were expressed as pg/ml. Levels of IL-12-p40

exceeding 150 pg/ml and of IFN- $\gamma$  exceeding 40 pg/ml were considered to be elevated. Leukocyte and platelet numbers were assessed according to standard techniques. Plasma protein was measured with a microhematocrite method. Heparinized blood was centrifuged for 10 minutes at 10,000-12,000 rpm<sup>(15)</sup>. The supernatant was analysed for protein content with a refractometer (Atago SPRN, Atago CO Ltd, Japan).

## Data analysis

The difference between groups with respect to age was assessed by the Anova test. Differences in levels of IL-12-p40 and IFN- $\gamma$  between cases and controls and between shock and normotensive patients were analysed by the Wilcoxon-Mann-Whitney (WMW) test. Differences in the proportion of elevated levels of IL-12 and IFN- $\gamma$  between cases and controls, and the distribution of gender between DF and DHF were analysed by the Chi-square test. Correlations between IL-12-p40 and IFN- $\gamma$ , respectively, and clinical and hemodynamic parameters were evaluated with the Spearman's correlation test. A two-tailed p-value of less than 0.05 was considered to represent a significant difference. All statistical calculations were done in SPSS 6.0 for Windows 95.

## Results

### Patients

During September 1995 - May 1996, 235 patients, admitted to the hospital with fever lasting for 2 to 7 days suggestive of dengue infection, were included in the study. Only 186 patients fulfilled the serological criteria (IgM with or without IgG) for dengue virus

infection. Seventy-one of these fulfilled the WHO criteria for dengue haemorrhagic fever (DHF) [22 cases for DHF-1 (11.8%), 20 cases for DHF-2 (10.8%), 18 cases for DHF-3 (9.7%) and 11 cases for DHF-4 (5.9%)]. The other patients (115) with positive serology were considered to suffer from dengue fever (DF) (61.8%). The mean age (year) of patients with DF was:  $8.15 \pm 3.15$  years; with DHF-1:  $9.73 \pm 3.15$  years; with DHF-2:  $8.50 \pm 3.28$  years; with DHF-3:  $8.39 \pm 3.58$  years; and with DHF-4:  $7.40 \pm 2.32$  years. The distribution of gender for DF was 51.8% male, 48.2% female; DHF-1: 68.2% male, 31.8% female; DHF-2: 40% male, 60% female; DHF-3: 33.3% male, 66.7% female; DHF-4: 54.5% male, 45.5% female. There was no statistically significant difference in age and gender distribution between groups (Anova:  $p=0.24$ ; Chi-square:  $p=0.2$ ).

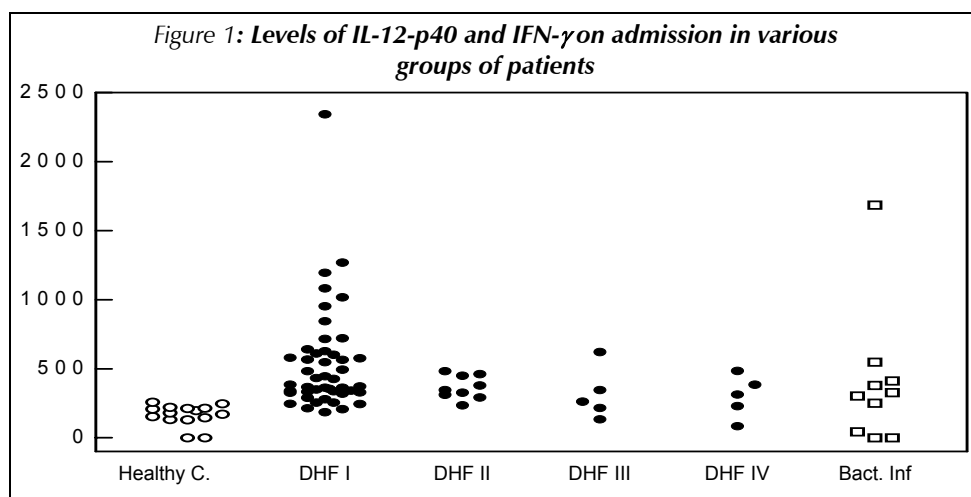
### Levels of IL-12-p40

Levels of IL-12-p40 in plasma samples obtained from 31 healthy children ranged from 139-1471 pg/ml, the majority of these children (96.8%) having elevated levels (Table 1, Figure 1). Plasma levels were elevated in 90% of the dengue virus infection patients with a range of <50-2550 pg/ml and a median of 322 pg/ml. The highest proportion of elevated IL-12-p40 levels was found in patients with DF (97.3%; range: 121-2550 pg/ml, median: 373 pg/ml), followed by DHF-1 (89.5%), DHF-2 (80%), DHF-3 (77.8%), and DHF-4 (54.5%). Thus, plasma levels of IL-12-p40 in the patients with DF or DHF were comparable with those in the apparently healthy children, as well as with those in the patients with bacterial infections (Table 1). IL-12-p40 levels did not fluctuate much during the course of the disease in the patients with DF or DHF (Table 2).

**Table 1:** Levels of IL-12-p40 and IFN- $\gamma$  on admission in various groups of patients

Group	Interleukin-12-p40 (pg/ml)			Interferon- $\gamma$ (pg/ml)		
	Mean no. of samples	Median (range)	% elevated	Mean no. of samples	Median (range)	% elevated
Dengue virus infection	180	322(<50-2550)*	90	184	<40(<40-3511) <sup>+</sup>	36.4
DF	112	373(121-2550)*	97.3	113	<40(<40-3511) <sup>+</sup>	44.2
DHF-I	19	235(128-1596)*	89.5	22	<40(<40-106)	36.4
DHF-II	20	255(89-519)	80	20	<40(<40-2177)	30
DHF-III	18	189(57-504)	77.8	18	<40(<40-349)	16.7
DHF-IV	11	209(<50-294)	54.5	11	<40(<40-<40)	0
Healthy children	31	373(139-1471)	96.8	31	<40(<40-114)	16.1
Bacterial infection	11	410(<50-984)*	81.8	11	<40(<40-443)	45.5

Wilcoxon-Mann Whitney test: \*: levels of IL-12-p40 comparable with healthy children ( $p > 0.05$ ); +: levels of IFN- $\gamma$  higher than those in healthy children ( $p < 0.01$ ).



C-: healthy children; C+: children with bacterial infections; DF: dengue fever, DHF I: dengue haemorrhagic fever I; DHF II: dengue haemorrhagic fever II; DHF III: dengue haemorrhagic fever III; DHF IV: dengue haemorrhagic fever IV, horizontal line indicates median.



### Relation of IL-12-p40 and IFN- $\gamma$ to other variables

A positive correlation between the plasma levels of IL-12-p40 with the plasma protein levels and a negative correlation with the haematocrit values was found. Plasma levels

of IFN- $\gamma$  correlated with the plasma protein levels. Also, a significant correlation between the levels of IL-12-p40 and of IFN- $\gamma$  and the presence of ascites was found (Table 4). In contrast, the platelet counts correlated inversely with the plasma levels of IL-12 and of IFN- $\gamma$  (Table 4).

**Table 2:** Course of IL-12-p40 and IFN- $\gamma$  in the dengue virus infection patients

Features	Interleukin-12 (pg/ml)			Interferon- $\gamma$ (pg/ml)		
	Mean no. of samples	Median (range)	% elevated	Mean no. of samples	Median (range)	% elevated
Admission	180	322(<50-2550)	90.4	184	<40(<40-3511)	36.4
1 day after admission	81	285(<50-3876)	82.7	82	<40(<40-911)	24.4
2 days after admission	35	223(56-1745)	80.0	34	<40(<40-392)	5.9
3, 4, 5,6 days after admission	27	212(113-531)	81.5	28	<40(<40-205)	10.7
Reconvalescence (7 days and later)	73	297(110-1933)	93.2	71	<40(<40-1350)	9.9

**Table 3:** IL-12-p40 and IFN- $\gamma$  in patients with shock versus normotensive patients

Group	Interleukin-12-p40 (pg/ml)			Interferon- $\gamma$ (pg/ml)		
	Mean no. of samples	median (range) %	elevated	Mean no. of samples	median (range) %	elevated
Shock patients	29	196(<50-504)	69	29	<40(<40-349)	10.3
Normotensive patients	151	345(89-2550)*	81.8	155	<40(<40-3511)+	41.3

Wilcoxon-Mann Whitney test: \*: levels of IL-12 in normotensive patients higher than those in patients with shock ( $p < 0.01$ ); +: levels of IFN- $\gamma$  in normotensive patients higher than those in patients with shock ( $p < 0.01$ ).

**Table 4:** Relation levels of IL-12-p40 and IFN- $\gamma$  to clinical, laboratory and haemodynamic variables

Parameters	Interleukin-12-p40 (pg/ml)			Interferon- $\gamma$ (pg/ml)		
	Mean no. of samples	correlation coefficient	p-value	Mean no. of samples	correlation coefficient	p-value
Leukocyte count	178	0.04	0.3	182	0.08	0.6
Plasma protein level	178	0.15	0.04	182	0.2	0.00
Platelet count	174	- 0.36	0.00	178	- 0.2	0.004
Presence of ascites	168	0.35	0.00	170	0.23	0.002
Hematocrit value	177	-0.25	0.001	181	-0.12	0.08
Presence of bradycardia	179	0.13	0.07	183	0.1	0.1

## Discussion

In this study we found elevated plasma levels of IL-12-p40 in the majority of patients and elevated plasma levels of IFN- $\gamma$  in a substantial number of patients with dengue virus infection when using cut-off values for normal Dutch adults. In vivo, IL-12 may occur in at least 2 different forms: as so-called p40 and p70 forms. The latter form consists of a 35 kD chain linked to a 40 kD polypeptide chain, and is bioactive<sup>(9)</sup>. The p40 form consists of the 40 kD chain only, and, except maybe for an inhibiting effect on the IL-12 receptor, is not bioactive<sup>(13)</sup>. Because IL-12-p40 circulates at much higher levels than the p70 form, its measurement is used as an indirect parameter for the production of bioactive IL-12. We also measured IL-12-p70 in some of our patients and found levels to be detectable in only a small part of them (data not shown). An important biological effect of IL-12 is the induction of IFN- $\gamma$  synthesis. IFN- $\gamma$  levels significantly correlated with IL-12-p40 levels,

supporting the notion that IL-12-p40 in our patients indeed reflected the production of bioactive IL-12.

IL-12 is produced by monocytes or macrophages stimulated by bacterial, parasitic or virus infections. In animal models, IL-12 is indeed released into circulation following a challenge with endotoxin<sup>(16,17)</sup>. Consistent herewith, the levels of IL-12-p40 are increased in the majority of children with meningococcal sepsis<sup>(18)</sup>. During some virus infections, such as those with lymphocyte choriomeningitis virus, IL-12 may impair cytotoxic T lymphocyte generation<sup>(19)</sup>. Our data do not allow for conclusions regarding the cellular source of IL-12, but one scenario is that it originated from monocytes or macrophages infected with dengue virus.

Surprisingly, the levels of IL-12-p40 were also elevated in most of the apparently healthy children. The relatively comparable levels of IL-12-p40 in patients with dengue



virus infection and the apparently healthy children in the outpatients department could be due to the fact that these children were not really healthy. They may have suffered from chronic conditions such as parasitic infestations, which is very common in the area where the study was done. If this is the case, there is no reason to presume that in the dengue patients this was otherwise and therefore there does not seem to be an important IL-12-p40 response in dengue infection. On the other hand, IFN- $\gamma$  levels were significantly higher in dengue patients than in the apparently healthy children. In another study also increased levels of IFN- $\gamma$  have been found in children with dengue infections as compared with healthy controls<sup>(20)</sup>. In a recent study IFN- $\gamma$  concentrations were measured in children with fever caused by dengue infection of less than 72 hours duration<sup>(21)</sup>. The duration of fever in dengue is typically 3-5 days and plasma leakage tends to occur at or around the time of defervescence. Therefore, this study design allowed for the assessment of plasma levels of this cytokine in a time period preceding the period of maximal plasma leakage<sup>(22)</sup>, in contrast to our study in which children were admitted to the hospital not before the third day of the disease and often later. In the above-mentioned study<sup>(21)</sup> the mean plasma IFN- $\gamma$  levels were significantly higher in children with dengue than those with nondengue febrile illness, but not different between patients with DHF and those with DF, but few patients with DHF 3 and none with DHF 4 were included. The day of peak IFN- $\gamma$  production for all patients occurred before or on the day of defervescence. Therefore, in our study, we may have missed peak levels, particularly since IFN- $\gamma$  has a short plasma half-life.

Nevertheless, IFN- $\gamma$  levels in our study correlated with the presence of ascites which is considered a marker of plasma leakage. In vitro, IFN- $\gamma$  can increase permeability in endothelial cell monolayers<sup>(23)</sup> and upregulates expression of TNF receptors on myeloid and epithelial cells, thereby rendering these cells more sensitive to the effects of TNF- $\alpha$ , which can also increase the permeability of endothelial cell monolayers<sup>(23,24)</sup>.

However, in our study, IFN- $\gamma$  concentrations (and also of IL-12-p40) were significantly lower in patients with dengue shock syndrome than in dengue patients without shock. This finding is not easy to explain, since plasma leakage is considered an important mechanism inducing shock. Although a positive correlation between IFN- $\gamma$  levels and protein levels and an inverse correlation between IFN- $\gamma$  and the haematocrit levels also does not support a role for IFN- $\gamma$  in inducing plasma leakage, this is in contrast with the finding that patients who developed ascites had higher levels of IFN- $\gamma$ . In summary, in our patients with dengue who were admitted to the hospital relatively late in the course of the disease (by which we may have missed early responses) we did not find an important IL-12-p40 response when compared to apparently healthy children, whereas IFN- $\gamma$  levels were significantly higher than in these negative controls. The study provided conflicting results as to the potential pathogenetic role of IFN- $\gamma$  in the induction of plasma leakage. Therefore, the data do not allow for firm conclusions on the role of both cytokines in the pathogenesis of dengue virus infection.

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# The Interdomain Region of Dengue NS5 Protein Interacts with NS3 and Host Proteins

by

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## Abstract

Although dengue virus genome replication occurs in the cytoplasm of infected cells, it has been shown that the NS5 protein (RNA-dependent RNA polymerase) is hyperphosphorylated at a late stage in infection and localized to the cell nucleus. A 37 amino acid sequence of NS5 (residues 369-405) was shown to contain a functional nuclear localization signal (NLS) that interacted with the cellular nuclear transport factor, importin  $\alpha/\beta$  heterodimer. Further studies using the yeast two-hybrid system revealed that the NS5 region (residues 320-368) immediately adjacent to the NLS contained an importin  $\beta$ -binding site that abuts or overlaps the binding site for the NS3 protein (protease/helicase). The importin  $\beta$ -binding site has also been shown to be a functional NLS (bNLS). Intriguingly, when both bNLS and NLS (residues 320-405) were present, the fused  $\beta$ -galactosidase protein did not accumulate in the nucleus. Here we provide a review of our studies on the NS5 interdomain region and compare it to other members of the Flavivirus genus in order to highlight the importance of this region as a possible target for developing broad-acting antiviral agent against dengue and other mechanistically-related viruses.

**Keywords:** NS5 protein, protein interactions, NS3 protein, nuclear localization signal.

## Introduction

The *Flaviviridae* family contains three genera, *Hepacivirus*, *Flavivirus* and *Pestivirus* that are small, enveloped, single-stranded (ss) and positive polarity RNA viruses. The dengue virus, of which there are four distinct

serotypes (DEN 1-4), belongs to the *Flavivirus* genus. The DEN-2 virus is the most prevalent and frequent cause of epidemics in many parts of the world. In Australia, dengue epidemics have occurred sporadically in the north-eastern part of the continent (North Queensland) and detailed

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accounts of epidemic outbreaks of dengue in this region date back to 1898<sup>(1)</sup>. Since the 1990s the frequency of outbreaks of dengue fever have escalated, mirroring the situation in the neighbouring regions and elsewhere in the world<sup>(2)</sup>. Although dengue virus is not endemic to North Queensland, the widespread prevalence of the mosquito vector, *Aedes aegypti*, results in small outbreaks (after introduction by international travellers) that are controlled by the excellent public health measures in Australia. However, the 1992/1993 dengue epidemic in the North Queensland towns of Townsville and Charters Towers was quite widespread and more than 1000 cases of dengue were reported. The DEN-2 strain TSV01 was isolated from a Townsville patient in 1993<sup>(3)</sup> and its complete nucleotide sequence has been obtained recently (Genbank accession no: AY037116).

The ~11 kb ss (+) RNA genome of dengue virus is capped at the 5' end but not poly-adenylated and upon uncoating, serves directly as a template for the synthesis of the virus proteins. A single translation initiation site leads to the production of a precursor polyprotein that is arranged NH3+-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COO-. Host signal peptidases and viral proteinases co-translationally and post-translationally process the polyprotein into at least 10 viral proteins; the three structural proteins C, prM and E that form the virion particle, and the seven non-structural proteins, NS1 to NS5, that function in the virus life cycle. The untranslated terminal regions account for less than 5% of the genome, and complementary elements in these regions form stem-loop structures and cyclization motifs that are important for the synthesis of new RNA<sup>(4)</sup>. In Flavivirus genome replication

the membrane-associated synthesis of minus-strand produces a dsRNA known as the replicative form (RF), which is the recycling template for the synthesis of new plus strand RNA. To achieve this, the viral proteins NS5 and NS3 act in concert through protein-protein interactions within the Replicative Complex (RC) that includes other viral NS proteins. The details of the interactions within the RC are poorly understood and our laboratory has chosen to focus on the protein-protein interactions of NS3 and NS5 using the observation that NS5 is localized to the nucleus<sup>(5)</sup> as a handle.

## Structure and function of NS5 and NS3

NS5, the largest of the ten flavivirus proteins at 104kDa (900 amino acid residues), is a multidomain protein with at least two domains that contain enzyme activities that are crucial for the replicative cycle of the virus. The N-terminal region is associated with the RNA capping reaction that puts a cap 1 structure ( $7^{\text{Me}}\text{G}^{5'}\text{-ppp}^{5'}\text{-N}^{\text{Me}}$ ) on the plus strand RNA genome and the C-terminal contains the eight highly conserved sequence motifs (I to VIII) that have been recognized in many RNA-dependent RNA polymerases (RdRPs) and includes the tripeptide "GDD" found in all polymerases (POL)<sup>(6,7)</sup>. Interestingly the capping activity involves three enzymatic steps, a 5'-terminal RNA triphosphatase, guanylyltransferase, and RNA methyltransferase. The first of these, 5'-terminal RNA triphosphatase activity, is contained within NS3 and the methyltransferase (MTase) activity has only recently been demonstrated for the first time in a recombinant truncated NS5 protein comprising residues 1-296. The 3D structure of the NS5<sub>MTase</sub> domain has been solved<sup>(6)</sup>.

Located between the two enzymatic domains is the region that we have characterized to be a "hot-spot" for protein interactions with cellular importin proteins and viral NS3<sup>(8,9,10)</sup> (Figure 1A).

NS3, is composed of 618 amino acids, plays a critical role in virus protein maturation and replication. The N-terminal one-third (167 amino acid residues) has the characteristic serine protease domain that requires NS2B for cleaving the polyprotein at NS2A-NS2B, NS2B-NS3, NS3-NS4A and NS4B-NS5 junctions. The remainder of NS3 forms the helicase domain which consists of nucleotide binding, nucleotide triphosphatase (NTPase) and RNA binding motifs. Previously, a truncated 50kDa fragment of NS3 amino terminal region (designated NS3') with a potential cleavage site located in the helicase domain<sup>(11,12)</sup> has been found in flavivirus-infected mammalian cells, but not in infected mosquito cells. A biological significance for the autolysis has not been established, although it has been speculated to regulate the helicase activity.

## Nuclear localisation of NS5

Whilst it is well known that the *Flavivirus* RNA replication occurs in the cytoplasm, a hyperphosphorylated form of dengue NS5 that does not interact with NS3 has been located in the cell nucleus<sup>(5)</sup>. Similar phosphorylation and nuclear localization of NS5 has been reported for another lymphotropic *Flavivirus*<sup>(13)</sup>, Yellow Fever virus, suggesting that these processes are probably functionally important in the virus life-cycle and perhaps in viral pathogenesis. Since the nuclear pore complex does not permit the entry by passive diffusion of proteins >45kDa, a short non-cleavable peptide sequence called the nuclear

localization signal (NLS) is required for the active nuclear import of large protein-protein complexes mediated by cytosolic NLS-binding proteins and other factors by a number of different pathways<sup>(8,10,14)</sup>.

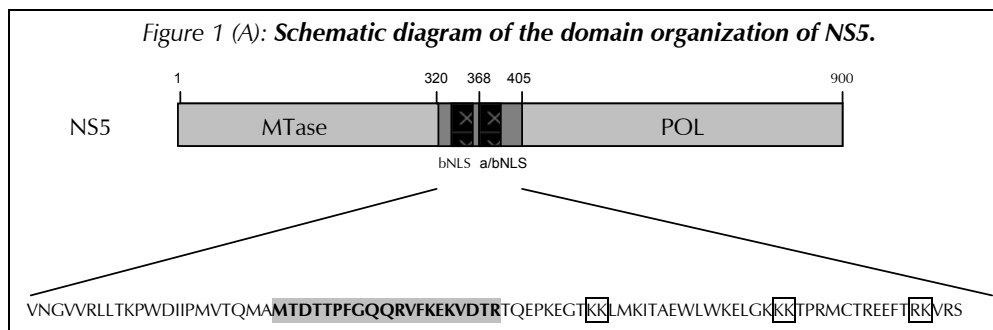
## Functional NLSs in NS5 and interaction with NS3

Since NS5 functions in the cytoplasm of infected cells we argued that the NLS must be masked at the early stage when the proteins are assembled into the RC. For this reason the best candidate for a functional NLS was predicted to occur within residues 369-405. We demonstrated both *in vivo* (microinjection) and *in vitro* that the 37 amino acid sequence from NS5 residues 369-405 when genetically fused to the normally cytoplasmic  $\beta$ -galactosidase ( $M_r \sim 500$ kDa as a tetramer) and fluorescently labelled (to render it visible by confocal laser scanning microscopy, CLSM), can function as a NLS<sup>(8)</sup>. NS5(369-405)- $\beta$ -gal contained two potential bipartite NLSs, so in order to narrow down the functional NLS, site-directed mutagenesis of the positive charge amino acid clusters (boxed in Figure 1A) as well as truncated constructs NS5 (369-391)- $\beta$ -gal and NS5(386-405)- $\beta$ -gal were constructed. *In vitro* studies with these constructs showed that the functional NLS that interacts with cellular importin  $\alpha/\beta$  heterodimer is within NS5 residues 369-389 which we refer to as a/bNLS<sup>(10)</sup>.

Previous evidence for the NS3-NS5 interaction includes the demonstration of complexes *in vivo* in dengue 2 infected monkey kidney (CV-1) cells and recombinant vaccinia virus co-infected HeLa cells and co-immunoprecipitation with antisera against NS3 or NS5, and also by the

binding of NS3 to His-tagged NS5 that was immobilized on Ni-NTA affinity beads<sup>(5)</sup>. In addition to demonstrating biochemically by pull-down assays that bacterially-expressed NS3 and NS5 can interact with each other, we used a genetic screen for protein-protein interaction, the yeast two-hybrid (Y2H) system, to show that the C-terminal region of NS3 (residues 303-618) interacted specifically with NS5 residues 320-368<sup>(9)</sup>. As this region of NS5 is N-terminal to the a/bNLS, we examined the interaction of various NS5 constructs with importin  $\alpha$  and importin  $\beta$  separately<sup>(9)</sup> and also as heterodimers using a yeast three-hybrid system (unpublished). These studies showed that NS5 (320-368) interacted strongly with importin  $\beta$  (Kd 23.5 nM) but not at all with importin  $\alpha/\beta$  heterodimer. Interestingly, NS3 competed with Importin  $\beta$  for the same site. Furthermore, we demonstrated that NS5(320-368)- $\beta$ -gal accumulated in the nucleus to the same extent as NS5 (369-391)- $\beta$ -gal or NS5(369-405)- $\beta$ -gal albeit at a slower rate<sup>(10)</sup>, indicating that a second functional NLS that we refer to as bNLS is located in the NS5 interdomain region.

The region of NS5 from residues 320 to 405 were compared in order to extend our experimental findings for DEN-2 sequences (Figure 1B). Interestingly, a 20 amino acid sequence within the bNLS is almost completely conserved amongst all the flaviviruses, including the cell fusing agent virus (CFAV) that is tentatively classified as a member of this family<sup>(10)</sup>. In a previous genetic study with Kunjin virus replicons, Khromykh and colleagues<sup>(15)</sup> speculated that this region may be important for NS3 binding and we confirmed this experimentally<sup>(9,10)</sup>. We showed by 3D modelling that within the 20 amino acid region is a short peptide that is very similar to the N-terminal peptide of importin  $\alpha$  that binds to importin  $\beta$  to form the heterodimer<sup>(10)</sup>. Furthermore, the functional relevance of the NS5 interdomain region and its *potential* value in producing rationally designed attenuated strains for use as vaccine was demonstrated by alanine scanning mutagenesis of different charged residue (within NS5 residues 320-405) in a full-length infectious clone of DEN-4, which showed that the region was critical for virus viability<sup>(16)</sup>.



The N-terminal methyl transferase (MTase) and C-terminal RNA-dependent-RNA polymerase domain (POL) separated by the interdomain region composed of bNLS and a/bNLS (see text) with amino acid numbers are indicated. The amino acid sequence of NS5 residues 320 to 405 is shown in single-letter amino acid code, with the bNLS in the shaded box and the three clusters of positively charged residues in clear boxes.

Figure 1 (B): Comparison of NS5 residues 320 to 405 of Dengue subtypes and various members of the *Flavivirus* genus.

TSV01	MVNGVVRLLTKPEWIIIPMTQAMTDITFFGQQRVFKEKVDTRQESKESTKLMKITAELWKELGKKK-TERMCTREEFTKRVRS
NGC	MVNGVVRLLTKPEWVVPMTQAMTDITFFGQQRVFKEKVDTRQESKESTKLMKITAELWKELGKKK-TERMCTREEFTKRVRS
Den1-WestPac	MVNGVVRLLTKPEWVVPMTQAMTDITFFGQQRVFKEKVDTRPKAKRSTAQIEVTARILWGFLSRNK-KPRICTRREEFTKRVRS
Den3-H87	MINGVVKLLTKPEWVVPMTQAMTDITFFGQQRVFKEKVDTRPRMPGTEKVMETAEWLWRTLGKNG-KPRICTRREEFTKRVRT
Den4-814669	MVNGVVKLLTKPEWVVPMTQAMTDITFFGQQRVFKEKVDTRTPOPKPTSMVTITANILWALGKKK-NPRICTRREEFTKRVRS
Yellow Fever	MVNGVVKLLTKPEWKLEEVTRAMTDITFFGQQRVFKEKVDTRAKDPACTEKILKVVNRILFRHLAREK-NPRICTRREEFTKRVRS
Banzi	MINGVVKLLSKPEWRIEENVINAMTDITFFGQQRVFKEKVDTRAPEEPOCTEIKVNVNQLFDYLGRTK-QPRICTRREEFTKRVRS
Edge Hill	MVNGVVKLLSKPEWRIEENVINAMTDITFFGQQRVFKEKVDTRAPPDPPPTSAIMEVNVNQLFDYLGRTK-QPRICTRREEFTKRVRS
West Nile	LVNGVVRLLSKPEWTTITNVITAMTDITFFGQQRVFKEKVDTRAPEEPECVIVNETTNILWAFIAREK-RPRMCSREEFTKRVNS
Kunjin	LVNGVVRLLSKPEWTTITNVITAMTDITFFGQQRVFKEKVDTRAPEEPECVIVNETTNILWAFIAREK-RPRMCSREEFTKRVNS
St. Louis	MVNGVVRLLSKPEWMTITNVITAMTDITFFGQQRVFKEKVDTRAPEEPLGVAQILVDVTTDLWDFVAREK-KPRVCTREEFTKRVNS
JEV	LVNGVVKLLSKPEWATIANVITAMTDITFFGQQRVFKEKVDTRAPEEPACAEVILNETTNILWAFIAREK-RPRMCSREEFTKRVNS
Powassan	LLINGVVKLLSKPEWVAREDVTRAMTDITFFGQQRVFKEKVDTRAQEQQPTETVILRAVSDWILEYLSRA-KVRMCTREEFTKRVRS
Langat	LLNGVVKLLSKPEWVAREDVTRAMTDITFFGQQRVFKEKVDTRAQEQQPTETVILRAVSDWILERVAKS-RPRMCSREEFTKRVRS
TBEV	LLINGVVKLLSKPEWVAREDVTRAMTDITFFGQQRVFKEKVDTRAQEQQPTETVILRAVSDWILERVAKS-KPRMCSREEFTKRVRS
MVEV	LVNGVVRLLSKPEWATIGVITAMTDITFFGQQRVFKEKVDTRAPEEPOCVITVDETINILWAFIAREK-KARICTRREEFTKRVNS
Louping ill	LVNGVVKLLSKPEWVAREDVTRAMTDITFFGQQRVFKEKVDTRAQEQQPTETVITRAVNDWILERVAKS-KPRMCSREEFTKRVRS
Sepik	MLNGIVKLLSKPEWKFESVITAMTDITFFGQQRVFKEKVDTRTPSSPPPTETIKSGHFWLVKHAREM-RPRICTRREEFTKRVNS
Rio Bravo	AINGVVKLLSKPEWQALSFVSTMTDITFFGQQRVFKEKVDTRAPEEPEKEVIVRKVFTILVKRLKGGGKIKCTKEEFINLVES
CFA	AVNPLVRKIMPEWTTITNVITAMTDITFFGQQRVFKEKVDTRVPPHPOHIVRNRTITKHFIRLFKNRNLPRILSKREEFTKRVNS

The residues highlighted in dark boxes indicate amino acid identity and those in light shaded boxes signify amino acid similarity for the sequences compared.

## Model for nuclear transport of NS5

A schematic model (Figure 2) for the series of events that may occur in a dengue virus-infected cell that leads to the transport of NS5 to the nucleus is proposed here.

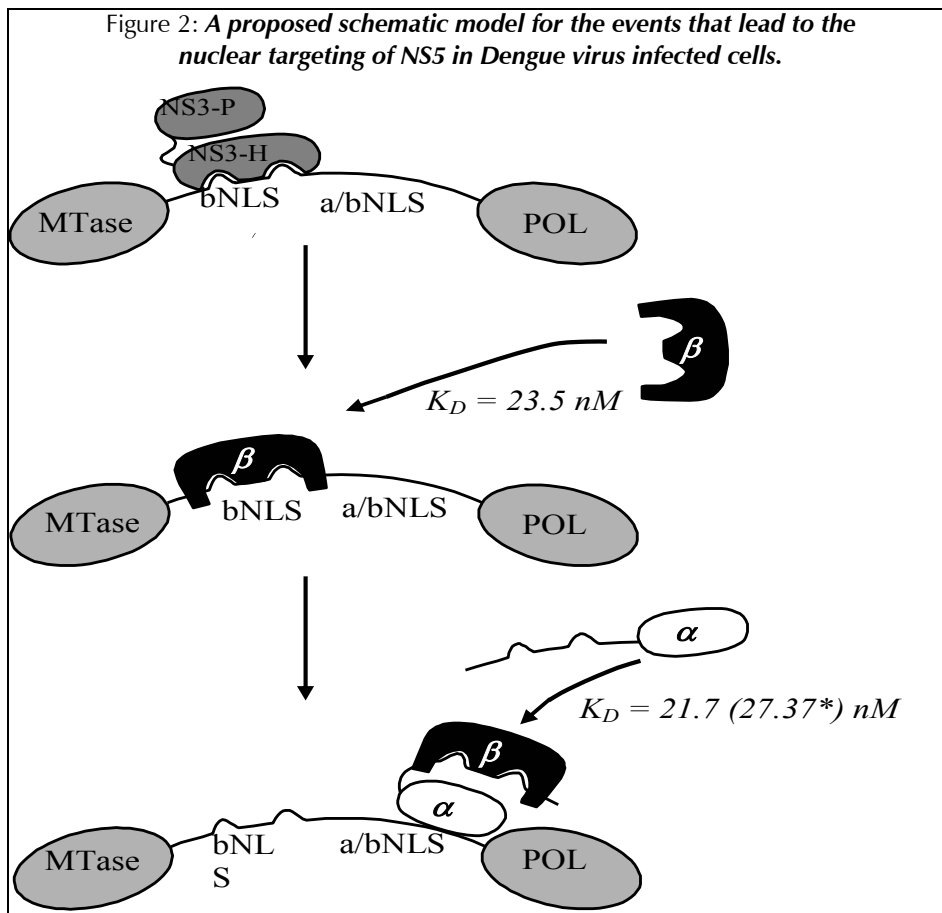
First, the RC is like a protein machine where virus and probably also some host proteins interact to replicate the viral genome. The interactions of NS3 and NS5 have been localized to the helicase domain of NS3 and the bNLS of NS5<sup>(9,10)</sup>. Mutations within bNLS as demonstrated by the changes to residues 356/357<sup>(16)</sup> affected full-length virus viability, probably because the critical interaction between NS3 and NS5 in the RC may have been disrupted. However, during the replication of wild-type virus the NS3 protein has been known to autoproteolyse within the helicase domain as mentioned earlier. This event probably leads to the

dislodgement of NS3 from bNLS by importin  $\beta$  since the affinity for this interaction is in the low nM range. Importin  $\beta$  is able to transport NS5 to the nucleus, but analogous to the situation in hepatitis C virus where NS5A is thought to sequester cellular importin proteins, we suggest that the transport of NS5 by importin  $\beta$  may also retain the protein in the cytoplasm. The slow rate of nuclear transport using bNLS compared to a/bNLS somewhat supports this notion<sup>(10)</sup>. The bound importin  $\beta$  probably recruits importin  $\alpha$ , and since the binding affinity of the N-terminal peptide of importin  $\alpha$  for importin  $\beta$  is in the very low nM range<sup>(17)</sup>, the formation of the importin heterodimer is promoted. The phosphorylation of NS5 at this stage leads to conformational change that exposes the a/b NLS sequence. This is then bound strongly by the importin  $\alpha/\beta$  heterodimer and located rapidly to the nucleus where it probably carries out functions that remain to be



discovered. Nevertheless, our characterization of the nuclear localization of dengue NS5 has led to a clear definition of the interdomain region of this multidomain

protein and opens the door to further studies that will be necessary for understanding the details of the interactions between NS3 and NS5.



NS5 is represented as two domains linked by the bNLS and a/bNLS region. The two humps in the bNLS indicate the conserved residues that are probably important for the importin  $\beta$  interaction<sup>(10)</sup>. NS3 is also indicated as a two domain protein with the helicase domain shown to interact with the bNLS region. The  $K_d$  values for protein interactions of Importin  $\beta$  with bNLS and Importin  $\alpha/\beta$  with the a/b NLS (the value in paranthesis is for interaction with NS5 residues 369-405). Once importin  $\beta$  binds the bNLS site after dislodgement of NS3 importin  $\alpha$  is recruited to the site. The importin  $\alpha/\beta$  heterodimer does not bind bNLS<sup>(10)</sup>, however it binds a/b NLS with nM affinity. This complex is then transported to the nucleus.

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# Study of Dengue Fever among Israeli Travellers to Thailand

by

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## Abstract

During 1995-2002, there were 149 cases among Israeli travellers, of which 110 (74%) were acquired in Thailand. The locations included Ko-Phangan (62%), Ko-Samui (20%), Ko-Tao (3%) and Ko-Phi Phi in Phuket area (15%). During the years of normal transmission in Thailand the cases were seen in the rainy season (July-November), but during the outbreak years (1998 and 2002) maximum cases occurred during the dry season (December-June). It is important for local health authorities to treat this source of information as a "sentinel source" for the detection of this emerging infectious disease.

**Keywords:** DF, travellers, Thailand, Israel.

## Introduction

Dengue fever (DF) has been one of the most important resurgent tropical diseases in the past 20 years, with an expanding geographical distribution of both the virus and the mosquito vector, increased frequency of epidemics, and the emergence of dengue haemorrhagic fever in new areas<sup>(1)</sup>. It is estimated that about 100 million cases of dengue fever and 250,000 cases of dengue haemorrhagic fever (DHF) occur annually<sup>(2)</sup>. The 1980s and 1990s saw a dramatic geographical expansion of

epidemic DHF from south-east Asia to the South Pacific Islands, the Caribbean and the American region<sup>(3)</sup>. South-east Asia and the Indian subcontinent, regions where dengue fever predominates, are popular tourist destinations. It is not surprising then, that DF may affect international travellers to these regions. These regions are popular sites for Israeli travellers with an estimation of more than 100,000 travellers annually.

The purpose of this paper is to address aspects of dengue fever among Israeli travellers who acquired DF in Thailand.

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## Materials and methods

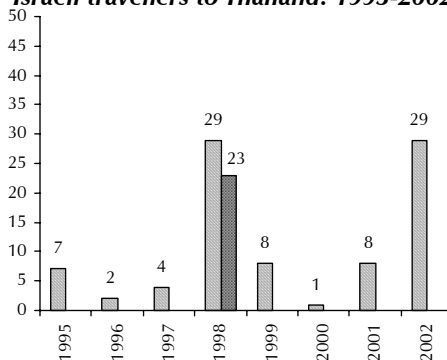
Patients seen at the Center for Geographic Medicine at Sheba Medical Center, Tel Hashomer, with a history of acute febrile illness were diagnosed as having dengue fever if they had a positive anti-dengue IgM test. Only IgM seropositive cases were included. The serology test was performed using a commercial enzyme-linked immunosorbent assay kit (PanBio, Queensland, Australia). This test was introduced in 1994, and the Sheba Medical Center remains the only place in Israel where it is routinely available.

During 1998, an Israeli physician was placed in Bangkok by a health insurance company. Cases diagnosed in Thailand during this year included Israeli travellers who had subscribed to this firm<sup>(4)</sup>. The service was based on prior subscription, and subscribers were eligible for free access to consultation provided by the Israeli physician on location. The availability of this free medical service encouraged subscribers to seek medical care, and it is unlikely that ill subscribers would not have called the service that was available at all hours of the day. These patients were also included in this study. The serology test for dengue IgM and IgG was performed by the physician at local hospitals using the PanBio enzyme-linked immunosorbent assay kit.

## Results

During the years 1995 through December 2002, 149 cases of dengue fever were diagnosed in travellers to Asia. Among them the majority (71%) acquired DF in Thailand; the rest of the cases did so in India, Laos, and Myanmar (Table 1).

**Figure 1: Number of dengue cases among Israeli travellers to Thailand: 1995-2002**



\* These cases were diagnosed in Bangkok, Thailand

Data regarding the disease pattern in travellers to Thailand are given in Figure 1. There was a peak during 1998, when altogether 52 Israelis were diagnosed as having acquired DF in Thailand. In 29 of these cases, DF was confirmed in Israel and in the other 23 cases, DF was confirmed by an Israeli physician who treated Israeli travellers in Bangkok. Another peak emerged during the year 2002 in which we confirmed 29 DF cases.

**Table 1: Distribution of Israeli dengue cases according to country of acquisition**

Country of acquisition	Number of patients No=149
Thailand	110
India	28
Myanmar (Burma)	3
Laos	2
Unknown	6

## Seasonality

Over the years, most cases were seen during the rainy season (July-November). Only during the years with high activity of the dengue virus did we see a high incidence during the dry season (December-June). As can be seen in Table 2, every 3-4 years there is a higher frequency of cases in the dry season. In 1995, there was an even distribution between the dry and rainy seasons (although the numbers were quite small). During 1998, 60% of the cases occurred in the dry season. This year (2002), we have seen 19 cases from Thailand from January to June (another case was acquired in Laos), and only 9 cases during the rainy season (68% vs. 32%, respectively). As can be seen in Table 2, in the other years we had a frequency of 2 out of 47 cases (4%) during the dry season.

**Table 2:** Distribution of dengue fever cases among Israeli travellers according to seasons

Year	Dry season (Dec-Jun)	Rainy season (Jul-Nov.)	Total Asia	THA
1995	5	6	11	7
1996	1	9	10	2
1997	0	18	18	4
1998	36	24	60	52
1999	1	8	9	8
2000	0	2	2	1
2001	0	10	10	8
2002	20	9	29	28

## Location

Almost all cases of DF were acquired on the islands of southern Thailand. The most common place was the gulf of Thailand where 85% of the cases had acquired infection in Ko-Phangan 62%, in Ko-Samui 20% and in Ko Tao 3%. The rest of the cases (15%) were acquired in Ko-Phi Phi in the Phuket area (Figure 2).



## Discussion

Changes in the global epidemiology of dengue fever have been observed in recent years in both North and South America as well as in the Pacific region and in south-east Asia<sup>(2,3)</sup>. This may be due to climatic changes and to the failure to control the mosquito vector<sup>(1,5,6)</sup>. In parallel, there has been a tremendous increase in the number of travellers worldwide, including to tropical areas. These parallel changes increase the likelihood of travellers being infected with the dengue virus.

Diagnosis of DF is a challenge in industrialized countries, since most physicians are less familiar with dengue fever

and its diagnosis. Moreover, diagnostic means for DF are lacking in many institutions. In addition, because of the short incubation period, most of the infected travellers will go through the sickness while in the endemic area. Thus, cases diagnosed in industrialized countries are probably only the tip of the iceberg.

The diagnosis of dengue virus infection is often based on commercially available ELISA test for serological assays. However, cross-reactivity exists between various flaviviruses such as Japanese encephalitis (JE) virus, yellow fever (YF) virus and the dengue virus. In endemic areas such as in south-east Asia it may be a challenge to differentiate between DF and JE when both circulate in the region<sup>(7)</sup>. However, JE is a very rare disease among travellers being a rural infection, and does therefore not constitute a real diagnostic challenge in travellers. But a substantial percentage of travellers to the tropics receive JE and YF vaccination prior to travelling, and the question arises whether these vaccines can interfere with dengue virus serology tests. Our previous study revealed that the IgG test yielded 11-17% and 15-44% positives in healthy travellers vaccinated against JE and YF, respectively. The dengue IgM was not found to cross-react with the vaccines<sup>(8)</sup>. This limits the ELISA-based IgG test for dengue diagnosis for use as an epidemiological tool to measure seroconversion rates among pre-vaccinated travellers after their stay in endemic areas. Thus, only symptomatic patients who returned from an endemic area with positive dengue IgM antibodies were included in our dengue studies.

Despite the strict criteria we used, DF appears to be a rather common disease among Israeli travellers to south-east Asia<sup>(4,9,10)</sup>. Thailand, which is an attractive tourist destination, is a major site for acquiring DF, mainly in the Gulf of Thailand<sup>(4,9)</sup>. The current study shows the same pattern, where 62% of the cases of the 2002 outbreak were acquired in Ko-Phangan. The high number of cases from Ko Phangan does not necessarily imply that there is an increased dengue virus activity in this island, since it can be due to the increased number of travellers to this attractive island. Unfortunately, we do not have a breakdown of the number of travellers to destinations within Thailand for a comparison of the attack rate. However, the itinerary of Israelis usually includes Bangkok area and the northern part of Thailand as well. Thus, the high number of cases from Ko-Phangan is highly suggestive for increased dengue activity.

The increase in dengue activity among our traveller population occurs in waves. During an outbreak, the change is not only an increase in the number of dengue cases during the regular dengue season, but rather a change in seasonality. We usually see dengue patients around the rainy season (July-November). However, during an outbreak there is a striking increase in dengue activity during the dry season (December-June) (Table 2). During the 1998 outbreak, the attack rate of cohort of 5,030 Israeli travellers to Thailand was 5.0/1000 (95% CI = 2.6-8.4/1000) in the dry season, and of 1.7/1000 (95% CI = 0.5-4.3/1000) in the rainy season<sup>(4)</sup>.

Data collected from travellers can indicate changes in the local epidemiology of infectious diseases, thus serving as a sentinel for emerging infectious diseases.

Regarding DF, travellers heralded the outbreaks of DF that occurred in 1998 and of the current year of 2002 (Figure 1). The Center of Diseases Control and Prevention (CDC, Atlanta) and the International Society of Travel Medicine (ISTM) keep a database – GeoSentinel – that collects data of morbidity among returning travellers from a network of 25 travel and tropical clinics around the world<sup>(11)</sup>. The information gathered by this kind of network may benefit both travellers and the host countries as well.

In a well-designed study which was published 15 years ago, dengue fever was

not mentioned as one of the hazards to the traveler<sup>(12)</sup>. The current data, our previous reports<sup>(4,9,10)</sup>, and reports by other authors<sup>(13,14)</sup>, indicate that dengue fever is a definite risk throughout the year for travellers. The attack rate is similar to the estimated rates for other frequent diseases of travellers such as hepatitis A without vaccine or malaria with no prophylaxis. This calls urgently for an efficacious and safe vaccine against this disease.

## Acknowledgement

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# The Features of Imported Dengue Fever Cases Confirmed at National Institute of Infectious Diseases Japan, during 2001<sup>+</sup>

by

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## Abstract

The demographic features of the dengue cases confirmed during 2001 at the National Institute of Infectious Diseases, Japan, were determined. Thirty-five cases were confirmed to be of dengue fever, 18 cases were male and 17 female. The youngest case was 19 years old and the oldest was 64 years old. Thirty-four cases were determined to be of primary infection, and one was secondary. Most of the dengue patients developed illness after returning from countries in Southeast and South Asia. In addition, two patients had visited Tahiti and one had visited Samoa before developing dengue fever. Dengue fever/dengue haemorrhagic fever is the infectious disease that should attract more attention in Japan.

**Keywords:** Dengue fever, imported cases, serodiagnosis, Japan.

## Introduction

Dengue fever/dengue haemorrhagic fever is one of the infectious diseases that all

physicians are required to report in Japan, according to the Japanese infectious disease control law. Dengue outbreaks occurred in

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+ Information generated on the circulation of dengue serotypes is equally important for 'travellers contact' countries. Introduction of new DEN serotype has been found to be a good predictive indicator for larger epidemics. During the present study, out of the 21 virus genome detections from countries of South-East and South Asia, 15 isolations belonged to DEN-1 and three each to DEN-2 and DEN-3. DEN-1 was the predominant virus circulating in the region during 2001. Besides, this information is important for DHF/DSS case management. In Thailand it has been established that the severity of DEN-1 and DEN-2 is associated with more plasma leakage, more shock and complication with fluid overload cases, whereas DEN-3 and DEN-4 severity is associated with hepatic dysfunction and encephalopathy. Advance stocking of DEN serotype-specific requirements in hospitals will result in better case management and help in lowering the case fatality rates. – Editor

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Osaka, Kobe, Hiroshima and Nagasaki from 1942 to 1945<sup>(1)</sup>. Dengue virus infection has not been epidemic in Japan since then, and there are no domestic dengue virus infections today. However, there have been dengue cases imported into Japan<sup>(2,3,4)</sup>.

Laboratory diagnosis is essential for the confirmation of dengue virus infection. We have performed laboratory diagnosis of dengue virus infection upon request from hospitals and clinics. The features of imported dengue cases that were confirmed at the National Institute of Infectious Diseases from 1985 to 2000 have been previously reported<sup>(2,3,4,5)</sup>.

## **Materials and methods**

Serum specimens were collected from dengue-suspected cases in clinics and hospitals, and sent to the Department of Virology 1, National Institute of Infectious Diseases, Tokyo, for laboratory diagnosis. In the present paper, we report the features of the dengue cases confirmed at our laboratory during 2001. Dengue virus infections were confirmed by IgM-capture enzyme-linked immunosorbent assay (ELISA), IgG-ELISA, rapid immunochromatographic test, haemagglutination inhibition (HI) test, and reverse transcriptase-polymerase chain reaction (RT-PCR) as previously reported<sup>(3,4)</sup>.

Commercial IgM-capture ELISA and IgG-ELISA (MRL, California, USA) and rapid immunochromatographic test (PanBio, East

Brisbane, Australia) were purchased and used for serodiagnosis. RT-PCR was performed as previously reported<sup>(6,7)</sup>. The primer sequences used to amplify each serotype of dengue viruses and target size were previously reported<sup>(6,7)</sup>. HI test was done on microtiter plate, using 4 haemagglutinin units of DEN-2 viral antigen as previously reported<sup>(2)</sup>.

## **Results**

The summary of dengue cases confirmed at our laboratory during 2001 is given in the Table. Blood samples from 76 suspected cases were tested and 35 were confirmed to be of dengue. All the cases were of dengue fever, and there was no case of dengue haemorrhagic fever. Of the 35 confirmed dengue fever cases, 18 were male and 17 female. The youngest case was 19 years old and the oldest 64 years old.

Most of the Japanese dengue patients developed illness after they had visited countries in Southeast and South Asia (Table), e.g. Indonesia (2), Thailand (8); Philippines (7); Cambodia (3), India (2), Tahiti (2), Viet Nam (1) and Samoa (1). There were nine patients who had visited more than one country. Thirty-four of the 35 cases were determined to be primary dengue virus infection based on antibody response. There was one patient (patient #27) who was determined to be of secondary dengue virus infection. This was the first secondary dengue case we experienced during the past five years.

**Table:** Demographic information of 35 dengue cases

No.	Age, Sex	Disease day	Type (PCR)	IgM-ELISA	IgG-ELISA	Countries of contact
1	33, F	9	-	+(1.1)**	+(7.3)	Indonesia
2	29, M	3	DEN-2	-(0.3)	-(0.7)	Indonesia
		7	-	+(3.2)	+(3.7)	
		9	-	+(3.6)	+(4.4)	
		14	-	+(3.7)	+(5.3)	
3	30, M	6	DEN-3	+(6.7)	-(0.6)	Thailand
		17	nd	+(15.4)	+(3.8)	Malaysia
4	30, M	6	DEN-1	-(0.2)	-(0.6)	India
		13	nd	+(4.0)	nd	Thailand
5	25, F	8	DEN-3	+(2.7)	+(5.9)	Cambodia
		10	nd	+(3.8)	+(7.9)	
		13	nd	+(4.1)	+(9.1)	
		17	nd	+(2.2)	+(9.5)	
6	49, M	7	DEN-1	+(1.5)	+(7.8)	Tahiti
		8	nd	+(2.3)	+(8.2)	
		13	nd	+(5.2)	+(9.3)	
7	56, M	18	-	+(2.5)	+(10.6)	Viet Nam
8	53, F	9	-	+(4.4)	+(9.2)	Philippines
		10	nd	+(3.8)	+(9.4)	
9	58, M	7	DEN-1	+(1.7)	+(5.8)	Philippines
		8	nd	+(2.8)	+(7.5)	
10	45, F	8	-	+(11.3)	+(2.4)	Philippines
11	23, M	4	-	-(0.8)	+(4.6)	Thailand
		5	-	+(1.4)	+(6.7)	Cambodia
		6	nd	+(2.2)	+(8.6)	Laos
		13	nd	+(8.2)	+(9.3)	
12	22, F	4	DEN-1	-(0.5)	-(1.0)	Philippines
		11	nd	+(4.1)	+(9.0)	
13	44, M	15	-	+(6.9)	+(8.1)	Thailand
		19	nd	+(6.7)	+(7.9)	
14	35, F	8	DEN-1	+(2.2)	+(6.8)	Singapore
		19	nd	+(2.4)	+(3.0)	Malaysia
						Thailand
						Indonesia
15	42, M	2	DEN-1	-(0.9)	-(0.7)	Samoa
		8	nd	+(5.0)	+(3.4)	
		14	nd	+(5.2)	+(1.6)	
		27	nd	+(3.4)	+(1.7)	
16	43, M	5	DEN-1	-(0.3)	+(2.2)	Thailand
		12	nd	+(3.6)	+(3.0)	Cambodia
						China

No.	Age, Sex	Disease day	Type (PCR)	IgM-ELISA	IgG-ELISA	Countries of contact
17	24, F	15	-	+(4.6)	+(1.5)	Thailand
		29	nd	+(3.2)	+(1.5)	Cambodia
18	22, M	5	DEN-1	-(0.2)	-(0.2)	Tahiti
		9	nd	+(2.5)	-(0.9)	
19	26, M	6	DEN-2	+(2.1)	+(1.1)	Thailand
		13	nd	+(3.1)	+(1.8)	
20	51, M	4	DEN-2	-(0.8)	-(0.1)	Philippines
		9	nd	+(4.0)	-(0.3)	
21	32, F	8	-	+(3.0)	+(1.3)	Cambodia
		16	-	+(3.5)	+(1.6)	
22	24, F	9	-	+(3.4)	+(1.7)	India
		23	nd	+(2.8)	+(1.7)	
23	28, F	7	-	+(3.7)	nd	Thailand
		8	nd	+(6.6)	-(0.4)	Cambodia
24	22, M	4	DEN-1	-(0.7)	-(0.1)	India
		16	nd	+(7.6)	+(10.2)	
25	26, F	8	DEN-1	+(5.7)	-(0.4)	Thailand
		23	nd	+(8.6)	+(6.5)	
26	28, F	7	DEN-1	+(4.9)	-(1.0)	Thailand
		16	nd	+(7.7)	+(1.8)	
27*	24, F	10	-	+(1.3)	+(2.0)	Philippines
		20	nd	+(1.1)	+(2.0)	
28	19, M	6	-	+(4.1)	-(1.0)	Thailand
		16	nd	+(6.5)	+(8.4)	
29	27, M	6	DEN-1	-(0.8)	-(0.1)	Cambodia
		13	nd	+(5.6)	+(1.3)	
30	21, F	5	DEN-3	-(0.7)	-(0.4)	Philippines
		6	nd	+(2.0)	-(0.9)	
		8	nd	+(3.8)	+(1.2)	
31	25, M	2	DEN-1	-(0.7)	-(0.2)	Thailand
		7	nd	+(6.8)	-(0.1)	
32	27, F	4	DEN-1	-(0.8)	-(0.2)	Thailand
		7	nd	+(5.1)	+(1.1)	
33	28, F	9	-	+(8.5)	+(7.9)	Thailand
34	25, F	7	DEN-1	+(4.9)	+(1.3)	Thailand
		29	nd	+(7.0)	+(7.0)	Laos
35	64, M	6	-	+(14.1)	+(6.9)	Myanmar
		16	nd	+(18.6)	+(8.7)	Thailand

\*: secondary infection: not detected      nd: not done

\*\*: Numbers in parentheses are index values. The values greater than 1.0 were determined to be positive.

## Discussion

Dengue virus infection is a serious cause of morbidity and mortality in most countries in the tropical and subtropical areas of the world<sup>(8,9)</sup>. Dengue is considered to be one of the most important infectious diseases in these regions. The cases that we confirmed to be of dengue at our laboratory accounted for only a part of the total imported cases in Japan. Nearly 5 million Japanese visit countries in the tropical and subtropical areas annually, and 2 million people visit Japan from these areas. Therefore, DF/DHF is one infectious disease that should attract more attention in Japan.

## Acknowledgement

We thank doctors of clinics and hospitals who provided serum samples for the laboratory diagnosis of dengue. This work was supported by grants from Research on Emerging and Re-emerging Infectious Diseases of the Ministry of Health and Welfare, Japan, and from Global Environment Research Coordination System, Ministry of the Environment, Japan, and by the Cooperative Research Grant 2001 (13-A-3) of the Institute of Tropical Medicine, Nagasaki University, Japan.

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# Use of Temephos for Control of Field Population of *Aedes aegypti* in Americana São Paulo, Brazil<sup>+</sup>

by

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## Abstract

The control of *Aedes aegypti* with 1% granulated formulation of temephos has been widely used in field control programmes in Brazil. The impact of temephos application was evaluated in Americana São Paulo, where an *Aedes* control programme was in place since 1991. Two areas with similar conditions of *Aedes* infestivity were selected. In the experimental area, temephos application @ 1 ppm at a frequency of three-month intervals and source reduction by communities were the methods used for control, whereas in the control area only source reduction was attempted. Monthly surveillance of larval population was undertaken using the larval indices, viz. Breteau Index (BI), Container Index (CI), and identification of *Aedes aegypti* types of breeding sites. Trends were also observed in relation to rainfall and minimum temperature. A covariance test was performed to compare the two different areas. Results indicated that the area subjected to temephos application presented similar levels of *Aedes aegypti* larval infestation as the untreated area. The larval infestation varied with the degree of rainfall but not with temperature. Positive breeding sites for *Aedes aegypti* more frequently encountered were plant vases, tanks and drums in the experimental area. A comprehensive operational research study is required to determine the causes of failure of temephos to suppress the *Aedes aegypti* population under an operational programme.

**Keywords:** Dengue, *Aedes aegypti*, temephos, control programme, Americana SP.

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## Introduction

Brazil, which achieved the eradication of *Aedes aegypti* in the 1950s, was re-infested in the 1970s and, by 1976, an extensive infestation of *Aedes aegypti* began in the seaports of Rio de Janeiro and Salvador. The species spread to other regions and, by the year 2000, vectors were reported in all Brazilian states<sup>(1)</sup>. In 1980, *Aedes aegypti* was identified in isolated focal points in the west of São Paulo state. Since then a combination of chemical and physical measures have been taken for its control<sup>(2,3)</sup>.

The main objective of this study was to evaluate the efficacy of temephos for the control of the larval population of *Aedes aegypti* as a part of a control programme's routine field actions over a ten-month period during 2000 and 2001 in a medium sized city in São Paulo state.

## Materials and methods

### Study area

Americana city (pop. 174,439) in São Paulo state, which reported occasional incidence of DF/DHF, led the local health authorities to intensify the *Aedes* control programme in 1991 based on source reduction and temephos application at three-month intervals, with community participation. Over the years the control programme stabilized.

Two areas were randomly chosen from a total of five, with similar socio-economic and *Aedes* infestation characteristics:

- Experimental area: 17,994 houses and 665 blocks

- Control area: 37,955 houses and 1775 blocks.

## Control activities

### Experimental area

- 1% temephos (1 ppm conc.) application in all "non-removable" wet containers, viz. drinking troughs, roof gutters, cement mixers, non-potable water cisterns and hollow trees at a frequency of three months.
- Source reduction of temporary water collection in "removable" containers, i.e. bottles, pots, cans, plastics, etc., by communities on a regular basis. Drinking water tanks were not treated.
- Health education by health staff.

### Control area

- Source reduction in removable containers by communities.
- Health education by health staff.

The whole city is visited by the 'Health Department Vector Control Team', comprising of 34 visitors and six supervisors, at three-month intervals.

### Data collection

Data was collected monthly to construct infestation indicators by random sampling in each study area (N=300). Index confidence intervals were calculated for N=300 samples. Types of positive breeding sites were recorded in monthly larval collection

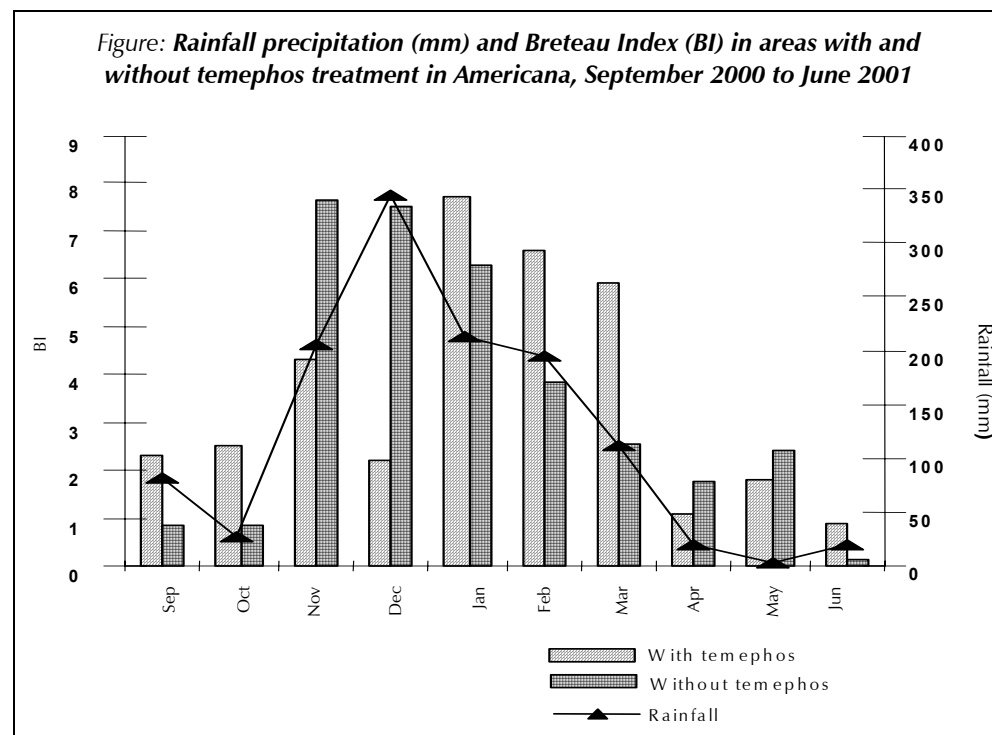
forms. The monthly average rainfall and minimum temperature data ( $^{\circ}\text{C}$ ) were provided by the local council. Rainfall was measured in millimetres of precipitation using standard collectors.

An analysis was made of ten months of larval infestation indices, viz. rainfall data and minimum temperature. The indicators obtained in the experimental and control areas were compared by covariance analysis, using Breteau Index (BI)\* and Container Index (CI)\*\* as the dependent variables; and larvicide use, minimum temperature, and rainfall as independent variables. The notifications of different types of breeding sites were compared by the *Student t* test,

assuming a significance level of 95% ( $\alpha = 0.05$ ).

## Results

The figure shows the Breteau Index (BI) and rainfall measurement in the study areas from September 2000 to June 2001. There were general similarities between both areas; however, larval density was slightly lower in the untreated area for most of the studied period. In December 2000, the *Aedes aegypti* infestation was substantially lower in the treated area. Data showed the seasonality of the infestation.



\* BI = (number of positive larval containers/number of buildings investigated) X 100

\*\* CI = (number of positive larval containers/number of water containers investigated) X 100



The Table gives estimates of the covariance analysis, demonstrating that larval infestation indices BI and CI did not vary with the use of temephos  $p=0.87$  and  $p=0.62$ , respectively. On the other hand, rainfall precipitation showed a significant impact on larval infestation,  $p=0.001$  for BI and  $p=0.02$  for CI; minimum temperature did not interfere with the detection rates of *Aedes aegypti* in this study ( $p=0.36$  for BI and  $p=0.19$  for CI). Minimum temperatures varied between 11.8 and 21.1°C.

Plant pot holders and vases were found to be the predominant breeding sites throughout this study. These positive types of *Aedes aegypti* containers were more frequent in the treated area ( $p=0.003$ ).

The *Aedes aegypti* larva-positive levels for permanent sites such as guttering, hollow trees, drinking troughs, bamboos, cement mixers, etc., were similar in both areas ( $p=0.13$ ).

**Table:** Covariance analysis of Breteau and Container Indices with temephos use, minimum temperature and rainfall, Americana SP, September 2000 to June 2001

	Independent Variables	F value	P
BI	Temephos	0.03	0.87
	Rainfall	16.11	0.001
	Minimum Temperature	0.89	0.36
CI	Temephos	0.26	0.62
	Rainfall	6.42	0.02
	Minimum Temperature	1.93	0.19

F value: Best value; P: Significant value

## Discussion

It must be remembered that these analyses were referenced in time and space. The numbers and different types of breeding sites and their normal uses varied from area to area.

The results of this study were contrary to the expectation that at least some residual effect of temephos was expected in the experimental area. However, the results suggested that there was no relevant impact of temephos application on *Aedes* larval prevalence.

One hypothesis is that the false complacency created by the larvicidal treatment contributed to the increased negligence in the physical elimination of breeding sites by communities as reflected by the presence of a high number of removable containers. The transitory effectiveness of temephos (around 3 weeks) as well as eventual overflowing of water and consequent dilution factors in non-removable containers added to the ineffectiveness of this treatment.

Vector resistance to temephos has been observed in the Caribbean and neighbouring regions<sup>(4,5,6)</sup>. Although there is no study available about temephos resistance status to *Aedes aegypti*, specifically in Americana, in the main regional city (Campinas) 40 km from the study area, bioassays showed 96.5% *Aedes aegypti* larval mortality after exposure to temephos<sup>(7,8)</sup>. This is less than the WHO limit (> 98%) at a dose concentration of 0.012 mg/l (ppm). Potential temephos resistance alone in the Campinas region does not seem to sufficiently explain the lack of success in the field.

A comprehensive study of the field routine is essential to identify the operational difficulties in the management of *Aedes aegypti* control programmes, such as insufficient interaction between professionals

and the community, ineffective implementation of temephos application in the field and lack of professional training in health education.

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# The Use of Ovitrap Baited with Hay Infusion as a Surveillance Tool for *Aedes aegypti* Mosquitoes in Cambodia

by

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## Abstract

This study was conducted to test (a) if a modified version of the CDC-enhanced ovitrap would attract more gravid female *Aedes aegypti* mosquitoes than standard ovitraps for more frequent monitoring of oviposition activity, and (b) the placement of ovitraps indoors or outdoors affected their performance. Paired ovitraps were placed in 25 strategically selected houses in Toul Kouk, a village on the outskirts of Phnom Penh, Cambodia's capital city. Each pair consisted of one ovitrap with 10% hay infusion and the other with plain tap water, one pair placed inside each house and the other outside the same house. Collections were made every other day for four weeks. The number of positive ovitraps was recorded and egg counts made.

Thirteen collections made over a 4-week period yielded a total of 7758 eggs, of which 5396 were collected in ovitraps with hay infusion. Ovitrap with hay infusion had a higher positivity (weekly range 15.56 – 54.55%) than ovitraps with plain water (weekly range 6.67 – 34.88%) ( $t = 4.92$ ;  $df\ 12$ ;  $p < 0.01$ ) and the mean number of eggs collected was significantly more in the enhanced ovitraps (415.07) than in ovitraps with plain water (181.69) ( $t = 7.33$ ;  $df\ 12$ ;  $p < 0.001$ ). Indoor and outdoor placement of ovitraps showed no significant differences in positivity or mean number of eggs collected either for infusion-baited traps ( $t = 0.25$ ;  $df\ 12$ ;  $p > 0.5$  and  $t = 0.06$ ;  $df\ 12$ ;  $p > 0.5$ , respectively) or for plain water traps ( $t = 1.97$ ;  $df\ 12$ ;  $0.05 < p < 0.1$  and  $t = 1.03$ ;  $df\ 12$ ;  $0.2 < p < 0.5$ , respectively).

Overall results indicate that, in the study site (a) hay infusion-baited ovitraps are a more sensitive indicator of the presence and numbers of *Aedes aegypti* than those with plain water and are suitable for frequent monitoring of *Aedes aegypti* oviposition activity, and (b) the location of ovitraps, indoors or outdoors, does not influence the performance of the traps.

**Keywords:** *Aedes aegypti*, enhanced ovitraps, surveillance, Cambodia.

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## Introduction

Most dengue control programmes rely on the use of *Aedes aegypti* larval indices as indicators of *Aedes aegypti* population densities for targeting control operations; however, they do not reflect adult mosquito population which has a great significance in the epidemiology of dengue infection.

A standardized CDC oviposition trap, developed by Fay & Eliason<sup>(1)</sup>, for use in *Aedes aegypti* surveillance, which indirectly determines the presence of adult gravid females, was improved by Reiter et al.<sup>(2)</sup> by the use of hay infusion rather than plain tap water as the medium. Paired ovitraps, one containing 100% hay infusion and the other 10% dilution of the same infusion, were found to greatly enhance the collection of *Aedes aegypti* eggs. Subsequent field studies in the Americas have also demonstrated the effectiveness of these CDC-enhanced ovitraps as sensitive surveillance tools<sup>(3,4)</sup>. A field evaluation of ovitraps with hay infusion was undertaken in Cambodia to determine their effectiveness as a surveillance tool.

## Materials and methods

A village, consisting of 50 houses, in Toul Kork, close to Phnom Penh, the capital city of Cambodia, was chosen as the study site. Twenty-five houses (every second house) were selected for the study.

Ovitraps (350 ml plastic cups, 91 mm in height and 75mm in diameter, painted black on the outside) were placed in pairs at each of the 25 houses - one pair outside and the other inside each house. Each pair comprised of one ovitrap with the 10% diluted hay infusion and the other with tap water. Ovitrap were lined with strips of

rough, absorbent paper (#76 seed germination paper, Extra Heavy Weight, Anchor Paper Co., Minneapolis, Minnesota) cut to a size that completely covered the inside surface of the ovitrap. Traps were prepared with 175 ml hay infusion or water and lined with appropriately labelled papers before transportation to the field. Hay infusion was made by steeping 125g of dried rice grass (*Oryza sativa*) in 15 litres of tap water in a tightly closed plastic garbage container for seven days. A new batch of hay infusion was started seven days in advance of each collection day. A 10% dilution of hay infusion was compared with tap water.

A total of 50 paired ovitraps were set out and exchanged between 0900-1200 hours, the time of lowest oviposition activity<sup>(5)</sup> on Mondays, Wednesdays and Fridays each week for four weeks. Ovitrap that had been emptied, removed or interfered with in any way were excluded from the final results. On collection, the hay infusion or water was discarded in the field and papers taken back to the laboratory in their respective cups. Papers were then removed from the cups and left to dry with the egg-bearing side face-up. Egg counts were made using a magnifying glass. Test results were statistically analysed by t-tests.

## Results

From 13 ovitrap collections over the four-week period, each using approximately 50 infusion-baited ovitraps and 50 plain water ovitraps, a total of 7758 *Aedes aegypti* eggs were collected (Table). The average number of eggs per infusion-baited ovitrap was 12.13 and for plain water ovitrap was 4.76.

**Table.** Total Number of Eggs Collected in Oviposition Traps

Location	Medium		Total
	Hay infusion	Plain water	
Inside house	2659	1292	3951
Outside house	2737	1070	3807
Total	5396	2362	7758

For ovitrap pairs placed inside houses, the difference in the percentage of positive ovitraps with and without hay infusion was significant (paired t test after arcsine transformation gave  $t = 3.26$ ;  $df\ 12$ ;  $0.005 < p < 0.01$ ) (Figure 1). Ovitrap with hay infusion inside houses yielded more eggs per trap than those with plain water (Figure 2). This difference was also significant ( $t = 5.86$ ;  $df\ 12$ ;  $p < 0.001$ ).

Similarly, for ovitrap pairs placed outside houses, the proportion of positive traps with hay infusion was higher than with plain water, and again, there was a significant difference ( $t = 4.41$ ;  $df\ 12$ ;  $p < 0.05$ ) (Figure 3). *Aedes aegypti* egg yields were higher in traps with hay infusion than in traps with plain water ( $t = 5.70$ ;  $df\ 12$ ;  $p < 0.001$ ) (Figure 4).

A comparison of the sensitivity of ovitraps with hay infusion only, both indoors and outdoors, showed no significant difference in positivity ( $t = 0.25$ ;  $df\ 12$ ;  $p > 0.5$ ) or mean number of eggs collected ( $t = 0.06$ ;  $df\ 12$ ;  $p > 0.5$ ). The same was observed in ovitraps with plain water for – positivity ( $t = 1.97$ ;  $df\ 12$ ;  $0.05 < p < 0.1$ ) and mean numbers of eggs ( $t = 1.03$ ;  $df\ 12$ ;  $0.2 < p < 0.5$ ). Thus, the placement of ovitraps, indoors or outdoors, did not significantly affect the results.

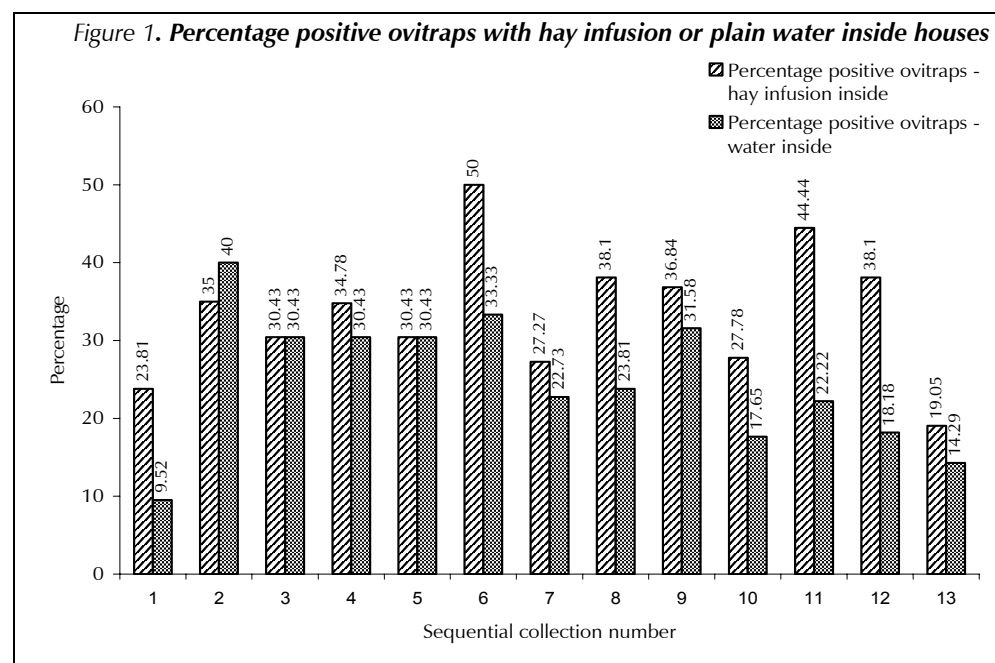


Figure 2. Mean numbers of eggs collected in ovitraps with hay infusion or plain water inside houses

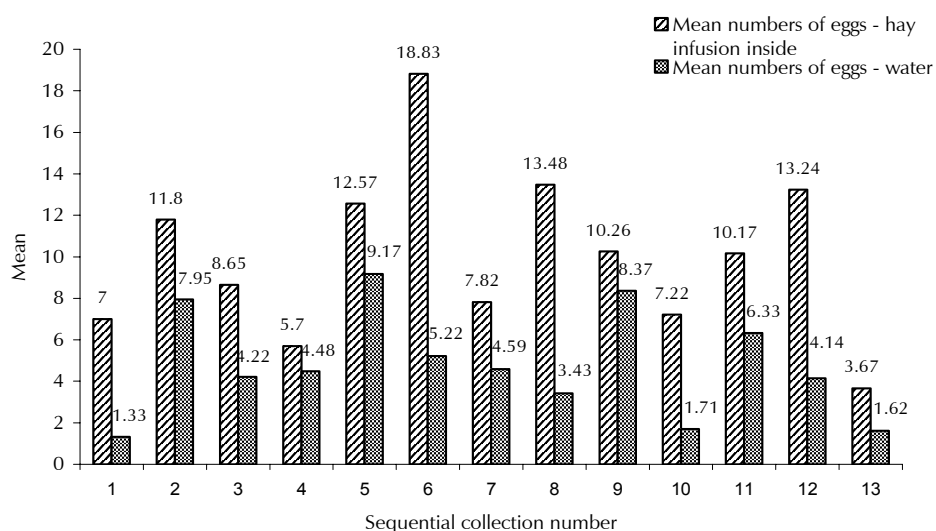
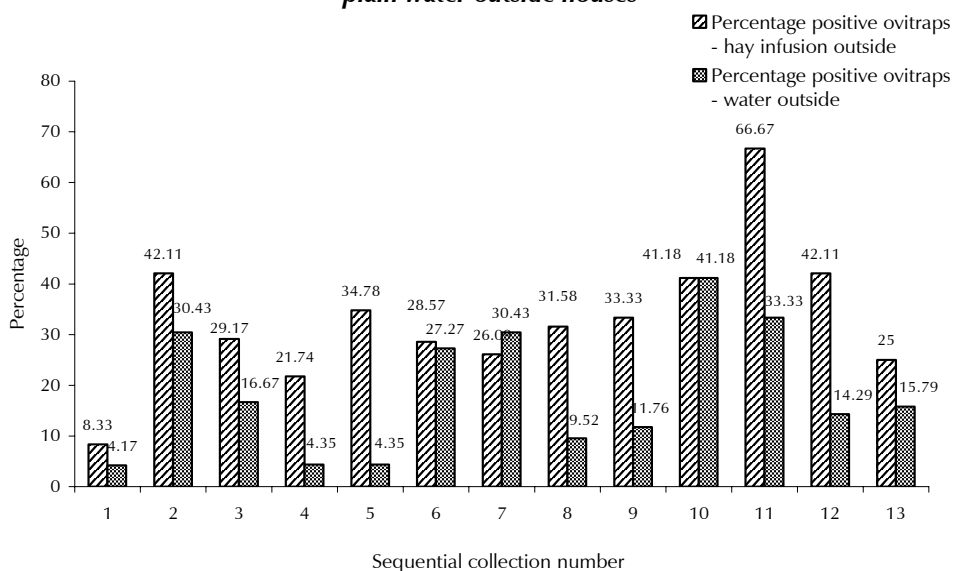
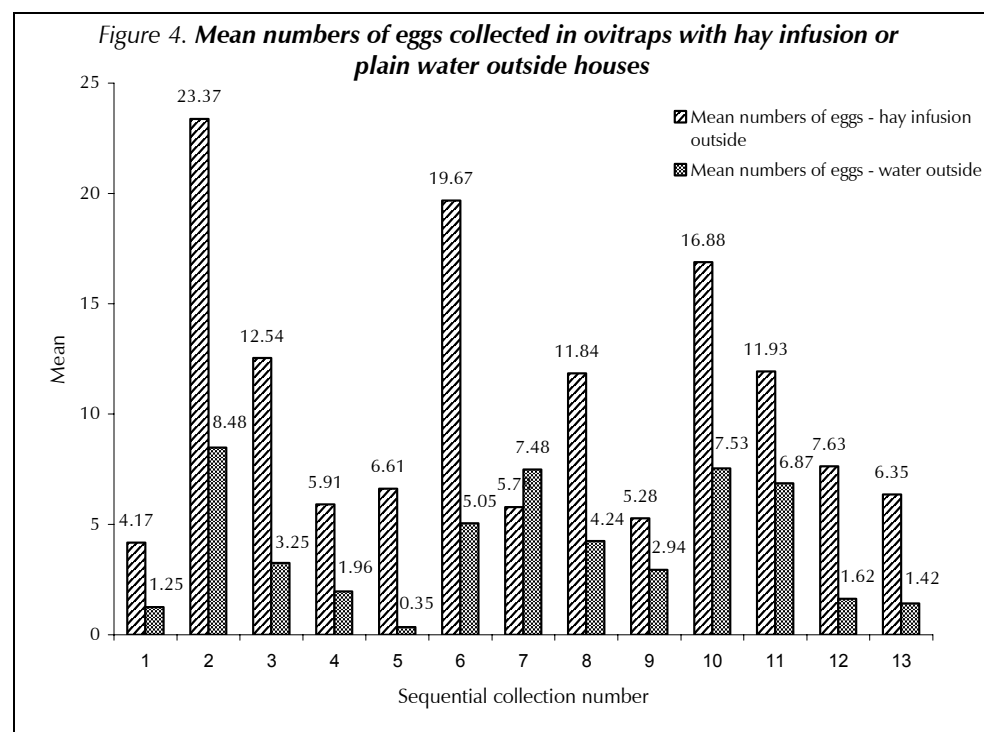


Figure 3. Percentage positive ovitraps with hay infusion or plain water outside houses





## Discussion

It is generally accepted that *Aedes aegypti* mosquitoes prefer to breed in clean water but the results of this study clearly corroborate that of other investigators, who demonstrated that *Aedes aegypti* mosquitoes deposited more eggs more frequently in ovitraps with hay infusion than in those with plain water<sup>(2,3)</sup>. Reiter et al.<sup>(2)</sup> investigated the oviposition response of *Aedes aegypti* to various dilutions of hay infusion in groups of ovitraps and found that ovitraps containing a 10% dilution collected the largest number of eggs. In their studies they also investigated several different paired ovitrap combinations of undiluted infusion (100%), 10% infusion and tap water. Their results showed that the

100%/10% pair gave the highest egg yield per collection. In the present study in Cambodia, the 10% infusion/plain water pair was used, which is one of the pairs included in the study of Reiter et al.<sup>(2)</sup>. The results of the present study are consistent with theirs in that within the 10% infusion/water pair the infusion received twice as many eggs as plain water. Although Reiter and colleagues recommend the use of the 100%/10% infusion paired combination, we suggest that for reasons of practicality, single ovitraps with 10% hay infusion are sufficient to enhance Cambodia's surveillance programme.

For our study we had ready access to a vehicle and the chosen study site was easily accessible from the laboratory so we were

able to transport 50 pre-prepared ovitrap pairs to 25 houses at the field site with two operators servicing them within one hour. Reiter et al.<sup>(2)</sup> suggest that two operators can service 80 ovitraps in a morning in an urban area without difficulty, but in an urban area in Cambodia where resources are limited and some areas are inaccessible by car, this may not be feasible. Often operators go to the field on motorbikes or on foot.

The operational implication is that the transportation of prepared ovitraps to the field would not be possible and, instead, operators would have to take bottles of infusion with them. If only one ovitrap, which requires hay infusion, is used, then that would lessen the load which operators would have to carry. Only 125g of grass is needed to make 15 litres of concentrated infusion which, when diluted to 10%, can service hundreds of ovitraps as, from our study, we found that eight litres of 10% hay infusion are required to service 50 ovitraps.

Results showed that there was no significant difference in the positivity and mean numbers of eggs between pairs of ovitraps placed indoors and outdoors. In both cases, ovitraps with hay infusion were more productive than those with plain water. For practical purposes it is easier for operators to place ovitraps outside rather than inside houses as this avoids having to deal with the problem of closed houses or householders not wanting field workers entering their homes. In the conduct of our study we were never able to recover all our traps on any of the collection days and this was in part due to householders not being at home to allow us access.

The other reason was that some ovitraps were either disturbed or overturned. Chadee et al.<sup>(6)</sup> suggested that although ovitraps placed at ground level do collect significant numbers of eggs, they found that in Trinidad, *Aedes aegypti* mosquitoes preferred oviposition sites up to 1.2 metres above the ground. If the same is found to be true in Cambodia, then placement of ovitraps off the ground would reduce the problem of ovitraps being disturbed by domestic animals.

The surveillance of adult female *Aedes aegypti* populations is the most useful indicator for assessing the impact of control programmes on disease transmission. In Cambodia, ovitraps are more practical surveillance tools as they are inexpensive and do not require any special skills on the part of the operator, and as we have shown, they also have the added advantage of being non-intrusive.

## **Acknowledgements**

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# Mechanisms of DDT and Permethrin Resistance in *Aedes aegypti* from Chiang Mai, Thailand

by

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## Abstract

Two strains of *Aedes aegypti*, one resistant to DDT but susceptible to permethrin (RdSp), and the other resistant to both DDT and permethrin (RdRp), were established in mosquitoes collected from Chiang Mai province, northern Thailand. Comparisons with a susceptible reference strain indicated that DDT resistance in both RdSp and RdRp strains was mainly due to an increase in DDTase activity. Similar moderate increases in cytochrome P450 levels were observed in the two resistant strains, hence this enzyme family may also play a role in DDT resistance. Glutathione S-transferase and esterase activities in the two resistant strains were similar and slightly higher than those of the susceptible strain, suggesting that neither enzyme group has a major role in permethrin resistance. The lack of an evident metabolic basis for the pyrethroid resistance in the RdRp strain suggests that nerve insensitivity may be present in this strain. The two mutations at residues reported to produce kdr resistance in other insects were not present, but some individuals from the permethrin resistant strain had an amino acid mutation at position 106 involving a valine to glycine mutation in the same segment 6 of domain II of the para sodium-channel gene, which may confer kdr-like resistance.

**Keywords:** *Aedes aegypti*, DDT, permethrin resistance, Chiang Mai, Thailand.

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## Introduction

*Aedes aegypti*, the primary vector of yellow fever, dengue and dengue haemorrhagic fever (DHF), is insecticide-resistant in numerous locations throughout the world<sup>(1)</sup>. DDT resistance is now widely distributed in *Aedes aegypti* throughout northern Thailand and it is also resistant to the pyrethroids, permethrin and deltamethrin, and the neopyrethroid etofenprox in many areas (P. Somboon, unpublished data).

The major mechanisms involved in DDT resistance in insects are increased metabolism of DDT by the glutathione S-transferase (GST) enzyme family and the insensitivity to inhibition of the voltage-gated sodium channel (known as *kdr*)<sup>(2)</sup>. As both DDT and pyrethroids act on the nervous system by modifying the gating kinetics of voltage-sensitive sodium channels, mutation in specific regions of this sodium channel can lead to binding failure with DDT and pyrethroids; therefore, cross-resistance between DDT and pyrethroids is common. In this paper, we applied biochemical and molecular assays to elucidate the resistance mechanisms in DDT-resistant and DDT/permethrin cross-resistant strains of *Aedes aegypti* from Northern Thailand.

## Materials and methods

### Mosquitoes

One-day old females, emerging from field-collected larvae of *Aedes aegypti* from Ban Pang Mai Dang, Mae Tang district, Chiang Mai province, Thailand, were exposed to 4% DDT-impregnated papers for 30 minutes in the WHO standard exposure tubes. These

females had <1% mortality, whereas those exposed to 0.25% permethrin for 60 min. gave 70% mortality. Single-family selections for several generations produced two strains of *Aedes aegypti*, one fully resistant to DDT ( $R^{dS^p}$ ) but susceptible to permethrin and the other resistant to both DDT and permethrin ( $R^{dR^p}$ ). These two strains were maintained under insecticide pressure for at least 10 generations before the resistance ratios were determined and the insects were harvested for biochemical and molecular assays. *Aedes aegypti* Rockefeller (Rock) strain was used as a reference-susceptible for comparisons.

### Bioassay and determination of $LT_{50}$

For all bioassay, four replicates (25 females per replicate) of 4 and 10 different exposure time periods for 4% DDT and 0.25% permethrin respectively were undertaken. Percentage mortalities were calculated for each exposure time and the mortality data were analysed on a log-time probit mortality regression using a computer programme provided by Dr C J Schofield, WHO, Geneva.

### Enzyme assays

One-day old females of each strain were used for enzyme or molecular assays. Each batch was homogenized in an appropriate buffer at 4°C and the 10,000 g supernatant was then determined for enzyme activities. The GST and DDTase activity were determined using the methods of Prapanthadara *et al.* 1996<sup>(3)</sup>. The esterase

activity was measured as described by Peiris & Hemingway 1990<sup>(4)</sup> with *p*-nitrophenyl acetate as the substrate<sup>(4)</sup>. The method for determination of insensitive acetylcholinesterase (IACHe) was as from French-Constant & Bonning 1989<sup>(5)</sup>. Mono-oxygenase activity was indirectly determined by measuring the different spectra of cytochrome P<sup>450</sup> in microsomal fraction by using a carbon monoxide trap.

Protein was assayed using the Bio-Rad protein reagent with bovine serum albumin as the standard protein.

The PCR fragments of the segment 6 domain II region of the sodium channel gene were obtained as described by Martinez-Torres *et al.* 1998<sup>(6)</sup>.

## Results

The LT<sub>50</sub>s of the R<sup>d</sup>Sp and R<sup>d</sup>Rp strains for DDT (Table 1) were 23- and 30-fold higher, respectively, than the LT<sub>50</sub> of the Rock strain. Log time-probit mortality lines for both R<sup>d</sup>Sp and R<sup>d</sup>Rp (Figure) were not linear for DDT (Chi square analysis  $P < 0.005$ ), indicating that resistance had not been selected to homogeneity. In contrast, the Chi square value for permethrin in both the R<sup>d</sup>Sp and R<sup>d</sup>Rp strain were non-significant which suggested that resistance to this insecticide had been selected to homogeneity.

One-day old adult female were used to determine all enzyme activities except cytochrome P<sub>450</sub>, where larvae were used in order to minimize pigment interference. Activities in each strain were determined using at least 20 batches of 10 mosquitoes and the mean activities  $\pm$  SD were calculated.

**Table 1:** Lethal time (minutes) that gave 50% mortality (LT<sub>50</sub>) in two insecticide-selected lines of *Aedes aegypti* compared with the susceptible Rock strain when exposed to 4% DDT or 0.25% permethrin on impregnated filter papers in a standard WHO tarsal contact assay

Strains	DDT			Permethrin		
	LT50	$\chi^2$	P	LT50	$\chi^2$	P
ROCK	17 (15-19)	36.9	NS	22 (21-24)	34.4	NS
R <sup>d</sup> Sp	390 (352-427)	27.9	< 0.001	18.607 (17.41-19.76)	5.8	NS
R <sup>d</sup> Rp	513 (466-559)	24.5	< 0.001	534 (502-566)	2.1	NS

**Table 2:** Comparison of mean GST, DDTase, cytochrome P450 and esterase activities in *Aedes aegypti* for the Rock, R<sup>d</sup>Sp and R<sup>d</sup>Rp strains

Strains	Rock	R <sup>d</sup> Sp	R <sup>d</sup> Rp
GST ( $\mu$ mole/min/mg)	0.22 $\pm$ 0.05	0.38 $\pm$ 0.09	0.37 $\pm$ 0.04
DDTase (nmole/mg)	3.23 $\pm$ 1.23	31.03 $\pm$ 13.88	26.85 $\pm$ 5.12
EST ( $\mu$ mole/min/mg)	0.2 $\pm$ 0.03	0.34 $\pm$ 0.07	0.26 $\pm$ 0.04
Cytochrome P <sup>450</sup> (nmole/mg)	0.07 $\pm$ 0.02	0.24 $\pm$ 0.02	0.28 $\pm$ 0.04

The enzyme activities are presented in Table 2. There was an ~10-fold increase in DDTase activity in the two resistant strains and a 4-fold increase in cytochrome P<sup>450</sup> activity compared to the Rock strain. The GST and esterase activities were slightly increased. There was no change in the sensitivity of acetylcholinesterase to inhibition by bendiocarb, in either resistant

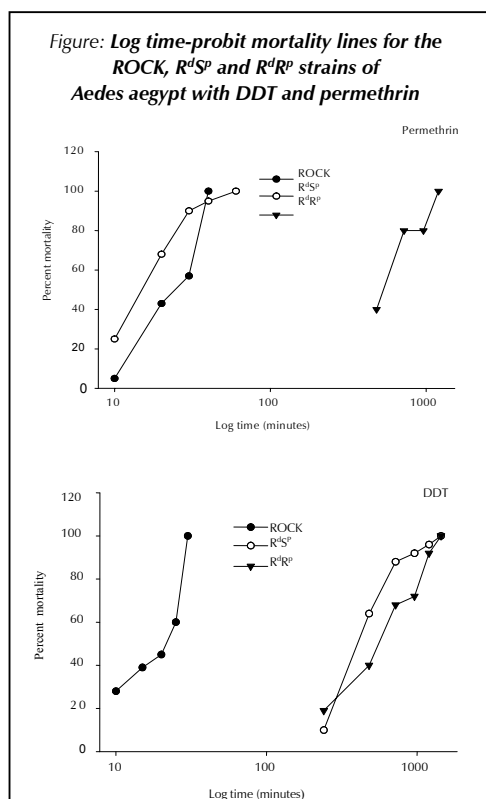
strain compared to the Rock susceptible. The levels of increased DDTase and cytochrome P<sup>450</sup> activities were very similar in the two resistant strains. This is in contrast to the difference in the DDT-resistance ratios. Sequencing of the S6 domain II region of the para-sodium channel gene demonstrated that the standard leucine to phenylalanine or leucine to serine mutation, which confers *kdr*-resistance in *An. gambiae*<sup>(6)</sup>, was not present in either resistant strain of *Aedes aegypti*. However, there was a valine to glycine mutation at position 106 in this domain in the 4 R<sup>d</sup>R<sup>p</sup> individuals sequenced. This mutation was not detected in the 4 R<sup>d</sup>S<sup>p</sup> or 20 Rock individuals sequenced.

## Discussion

We found that DDT resistance in both R<sup>d</sup>S<sup>p</sup> and R<sup>d</sup>R<sup>p</sup> strains was due to increased DDTase activity and cytochrome P<sup>450</sup> content whereas permethrin resistance in the R<sup>d</sup>R<sup>p</sup> strain probably involved a non-metabolic *kdr* mechanism. This *kdr* should also generate cross-resistance to DDT and would explain the greater resistance ratio to DDT in R<sup>d</sup>R<sup>p</sup> as compared to the R<sup>d</sup>S<sup>p</sup> strain.

In earlier reports of DDT and pyrethroid resistance in *Aedes aegypti*, selection of a resistant strain with DDT generated moderate resistance to pyrethroids, whereas selection with permethrin resulted in strong resistance to both permethrin and DDT<sup>(7)</sup>. Our results suggest that DDTase/cytochrome P<sup>450</sup>-based DDT-resistance does not confer cross-resistance to pyrethroids. However, the *kdr*-based pyrethroid resistance mechanism probably increases the DDT-resistance levels.

In conclusion, our study suggested that DDT resistance was due to multiple factors. DDT selection, as shown by our results and others<sup>(8)</sup>, often generates GST-based metabolic resistance which does not usually confer cross-resistance to other insecticides. This should be considered before a policy of abolishing DDT use for susceptible mosquito vectors is implemented. Although the persistence of DDT residues in the environment are well documented, making it unsuitable for large-scale spraying, limited use of this insecticide as an indoor spray may still be a good method of mosquito vector control in many countries.



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# Effect of Midgut Bacterial Flora of *Aedes aegypti* on the Susceptibility of Mosquitoes to Dengue Viruses

by

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## Abstract

*Aedes aegypti* mosquitoes collected from the field were surface sterilized and microbial isolations were made from the midguts. Isolates belonging to various genera were obtained, of which the one most abundant isolate was *Aeromonas culicicola*. This was first isolated and described as a new species from *Culex quinquefasciatus* mosquitoes and during this study frequent isolations of this bacteria were also obtained from *Aedes aegypti* mosquitoes. It was selected to study its effect on the susceptibility of *Aedes aegypti* mosquitoes to dengue (DEN) viruses. The isolate was incorporated in the blood meal of mosquitoes supplemented with the DEN virus. This resulted in the increased susceptibility of mosquitoes to the DEN virus. To further confirm this observation the experiments were repeated with *E. coli*, which also resulted in an increased susceptibility of mosquitoes to virus. To further investigate the effect of isolate on the susceptibility of mosquitoes to the virus, mosquitoes were treated with antibiotics to reduce the internal flora of mosquitoes. The treatment of antibiotic and incorporation of bacterial isolates in the blood meal did not show such an increase in the susceptibility of mosquitoes to the virus. The results suggest that there is a possibility that the quality of water in which *Aedes aegypti* mosquitoes breed and the bacterial flora they carry in their midgut might also have a role in determining their susceptibility to the virus.

**Keywords:** *Aedes aegypti*, *Aeromonas culicicola*, antibiotics, dengue virus, midgut flora, susceptibility.

## Introduction

Mosquitoes serve as the obligate intermediate hosts for numerous diseases that collectively are a major cause of human mortality and morbidity worldwide. In India, *Aedes aegypti* is considered to be an important vector of dengue<sup>(1)</sup>. Mosquitoes

show varied susceptibility to different pathogens that they transmit. Recently, it has been shown that mosquitoes offer both cellular and humoral responses to pathogens. There has been very little work done to understand such responses in mosquitoes with regard to arboviruses. The susceptibility of mosquitoes to arboviruses is

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considered as quantitative trait loci and various intrinsic and extrinsic factors, including genetic factors, have been ascribed to this<sup>(1)</sup>.

Mosquitoes harbour a large number of bacteria in their midgut. The midgut barrier is considered to be one of the major factors which governs the susceptibility of mosquitoes to arboviruses. Any pathogen that enters a mosquito through blood meal comes in contact with the midgut where it interacts with the epithelial cells and the resident bacteria of the midgut. There is very little information available on the effect of the resident microbial flora on the susceptibility of mosquitoes to viruses. It had been shown that certain antibiotics reduced the bacterial flora in the mosquito midgut, which in turn increases the infectivity of *Plasmodium falciparum* in the *Anopheles* mosquitoes<sup>(2)</sup>. Such effects have not been investigated for the susceptibility of mosquitoes towards the viruses. Therefore, the present study was conducted to understand the effect of bacteria on the susceptibility of mosquitoes towards the viruses. The most abundant and frequent bacterial isolate obtained from *Aedes aegypti* was studied to determine its effect on the susceptibility of mosquitoes to the dengue virus.

## Materials and methods

### Mosquito species

The *Aedes aegypti* mosquito species used in the experiments were obtained from laboratory colonies maintained at the National Institute of Virology, Pune. The mosquitoes were maintained at a temperature of 28°C ( $\pm$  2°C) and relative humidity of 80 ( $\pm$  5%).

## Antibiotics treatment

The resident gut flora of the *Aedes aegypti* mosquitoes was cleared using the protocol described by Toure et al<sup>(3)</sup>. Briefly, the cages were wiped with 70% alcohol before placing the pupae. Emerging adult mosquitoes were fed daily with a mixture of penicillin (10 microgram/ml), streptomycin (10 microgram/ml) and gentamycin (15 microgram/ml) in a 10% sterile sucrose solution on sterile cotton balls for three consecutive days. Sugar pads were removed and mosquitoes were starved for 24 hours before receiving blood meal containing bacterial isolate. After feeding, mosquitoes were given 10% sterile sugar solution on sterile cotton balls daily till they were dissected.

### Virus strain

Dengue-2 (DEN-2) virus strain (9012384) was isolated from a patient suffering from dengue haemorrhagic fever during an epidemic, which occurred at Chirimiri, India. Viral stocks used for experiments were prepared in mice.

### Bacterial strains

*E. coli* DH 5 alpha was used as control. *Aeromonas culicicola* MTCC 3249 was isolated earlier from the midgut of *Culex quinquefasciatus*<sup>(4)</sup>.

### Infection of mosquitoes through membrane

Dilutions of DEN virus were made in defibrinated white leghorn fowl blood. Four to five-days-old female *Aedes aegypti* were fed on the DEN-infected blood through an artificial membrane, Parafilm (American



National Can, Greenwich, CT 06836, U.S.A.) as described by Harada, Matsuoka & Suguri<sup>(5)</sup>. After feeding, mosquitoes were maintained for 10 days on 10% glucose solution in distilled water. The presence of antigen was checked after 10 days.

### Detection of virus in mosquitoes

Detection of the DEN viral antigen in the head squashes of the mosquitoes was done on the tenth post-infection (PI) day using the indirect immunofluorescence antibodies (IFA) technique<sup>(6)</sup>.

## Results and discussions

### Effect of bacterial isolates on the susceptibility of mosquitoes to viruses

Mosquitoes were fed with blood meal, which contained the DEN virus and bacterial isolate *Aeromonas culicicola* or *E. coli*. The presence of the viral antigen was determined on the tenth day. Similar experiments were repeated where mosquitoes were treated with antibiotics so as to eliminate the normal bacterial flora of the midgut. They were treated with antibiotics as mentioned earlier. In both the experiments, control was the batch of mosquitoes which did not receive the bacterial isolate in the blood meal containing the respective virus.

### Effect of bacterial isolate and *E. coli* on susceptibility of *Aedes aegypti* to DEN virus

The untreated *Aedes aegypti* carrying normal gut flora, when infected orally with the dengue virus, showed that 12.5% of the

mosquitoes were positive for the antigen in head squashes on the tenth day. There was more than a two-fold increase in the overall susceptibility to the dengue virus with the incorporation of bacterial isolate and *E. coli*. However, the increase in susceptibility was not seen when the antibiotic-treated mosquitoes were used (Table).

**Table:** Susceptibility of *Aedes aegypti* mosquitoes to dengue virus

	Percent mosquitoes positive (SD)	Fold increase in susceptibility
<b>Untreated</b>		
A. Control	12.5 (4.2)	—
B. <i>Aeromonas culicicola</i>	27.8 (9.6)	2.2
C. <i>E. coli</i>	27.8 (4.8)	2.2
<b>Antibiotics treated</b>		
A. Control	15.3 (2.4)	—
B. <i>Aeromonas culicicola</i>	9.7 (6.4)	—
C. <i>E. coli</i>	13.9 (4.8)	—

Number of bacilli used; B 4.2x10<sup>7</sup>/ml; C: 9x10<sup>7</sup>/ml  
 Post-feeding titre range in three replicates: 5.5 – 6.0 log  
 MID<sub>50</sub>/0.02ml mouse i.c.  
 In batch 2, mosquitoes were treated with antibiotics.  
 Average data of three replicates

[The mosquitoes were fed with bacterial isolate during the infection with dengue virus by oral feeding with blood. The susceptibility of mosquitoes to the virus was checked by the presence of antigens in the head squashes using immunofluorescence assays]

Recently, it has been shown that the dengue viral entry through adsorption and penetration leading to infection is accomplished within two hours and

carbohydrate residues may contribute to binding and penetration of the virus into the mosquito cells<sup>(7)</sup>. It is known that the entry of arboviruses into the gut cells is receptor-mediated. However, in the case of mosquitoes when they take blood meal, the formation of the peritrophic membrane starts within 20-30 min, which later on surrounds the blood bolus. Therefore, it is assumed that the entry of virus particles into the midgut epithelial cells must take place before the formation of the peritrophic membrane. In the orally-infected mosquitoes, soon after the blood meal the activity of the proteolytic enzymes starts, which also destroy the virus particles present in the blood bolus.

Earlier, it had been reported that *Cx. bitaeniorhynchus* treated with tetracycline showed an increased susceptibility of mosquitoes to the Japanese encephalitis (JE) virus<sup>(8)</sup>. The larvae of this mosquito species are found in slow running or stagnant water bodies and feed only on algae at immature stages. It is presumed that these mosquitoes have heavy resident microbial flora in the gut, which helps them in the digestion of algae. The treatment of antibiotics reduces their survival and also reduces the heavy load of normal gut flora. This probably increased the probability of the JE virus particles to bind to the receptors, which in normal course compete or block these receptors. In the past, similar observations had been made by Beier et al<sup>(9)</sup> that the reduction of normal bacterial flora in the mosquito midgut increased the *Plasmodium falciparum* infection rates.

It has also been reported that the incorporation of bacterial flora through the

blood meal get established in the midgut as resident bacteria<sup>(3)</sup>. In the present work when bacterial isolates were incorporated in the blood meal of *Aedes aegypti* mosquitoes supplemented with dengue virus, they produced a significant change in their susceptibility to the DEN viruses, of which they are a natural vector. *Aedes aegypti* mosquitoes are fresh-water breeders and are likely to have less load of microbial flora. The incorporation of these isolates has produced a very high change in their susceptibility to the DEN virus.

It has been shown recently that certain species of mosquitoes produce a defense response when challenged with bacterial or protozoal pathogens<sup>(10)</sup>. At the present juncture it is difficult to explain whether the differences in the susceptibility observed by the incorporation of isolates caused different degrees of defense responses, which might result in an altered susceptibility of mosquitoes to viruses. However, the data suggest that there is a possibility that the quality of water in which mosquitoes breed and the bacterial flora they carry in their midgut might also have some cumulative effect on the various intrinsic and extrinsic factors which contribute to determining their susceptibility to the virus.

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# Study of Child-Invented Health Educational Games on Dengue Fever

by

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## Abstract

The study's goal was to demonstrate the ability of an eight-year-old child to create educational games on the topic of dengue fever control. A naturalistic descriptive case study method was employed. The child had two dengue fever educational game creation activities. The study demonstrated that a child could develop functional games related to dengue fever control. The study, however, revealed knowledge gaps, and mixed methods for dengue fever-related mosquito control. The games' construction was consistent with the child's cognitive level. The case study revealed that a child-centred educational game creation may be both diagnostic for a child's topical knowledge and cognitive development, but also serve as a learning tool for children. This activity may also be an informational tool for formative research for dengue fever control.

**Keywords:** Dengue fever, children's educational dengue games, cognitive, informational tool, dengue fever control.

## Introduction

Elementary school-age children have demonstrated abilities in the invention of a wide range of games to cover such topics as general science<sup>(1)</sup>, social studies<sup>(1,2)</sup>, mathematics<sup>(1,2)</sup> and environmental studies<sup>(3)</sup>. Child-invented games tended to increase in complexity with rules and content as children increased grade levels from first grade through third grade in one study<sup>(1)</sup>.

Children's invented games provide a host of potential benefits. Previous studies on children's invented games described the following benefits: promotion of child-centred learning, problem-solving and cooperation, in-depth learning of specific topics, increased confidence in learning, an assessment of children's cognitive processes, promotion of organization in learning, and learning through fun activities<sup>(1,2,3)</sup>.

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The results of previous studies in child-invented games supported the theoretical approaches of Piaget in the importance of active learning, cognitive stage appropriate learning and the value of games in child-centred learning<sup>(4)</sup>. The previous studies also supported the importance of play or the fun element in learning<sup>(5,6)</sup>.

A child-created educational game may serve as a diagnostic tool for content understanding and learning/cognitive gaps for key health issues and problems. Therefore, this study seeks to examine a child's invention of educational games related to the problem of dengue fever control. This study will examine child-invented games as possible educational materials for children and as potential diagnostic tools for content, understanding and also cognitive/learning gaps related to the dengue problem. The study will also examine these issues through progressively increased pre-game creation instructions, from the first game creation to the second game creation.

## Background and significance of dengue

Dengue fever and its more severe form dengue haemorrhagic fever are rapidly increasing around the world. Nearly half of the earth's population lives within the dengue zone. Though primarily found in the tropics, indigenous dengue cases have recently been diagnosed in all continents except Europe. This caused Gubler to state: "Dengue fever is currently the most important arbovirus disease of humans<sup>(7)</sup>".

The number of globally reported dengue cases has nearly doubled in the past

decade<sup>(8)</sup>. Dengue has re-emerged after scores of years of absence in such locations as Texas, USA, on the North American mainland<sup>(9)</sup>, and Hawaii, USA, in the Pacific<sup>(10)</sup>.

The Philippines has seen a rapid rise in dengue cases. In 1989, there were approximately 3 dengue cases per 100,000 pop. By 1998, it had jumped to over 45 cases per 100,000 pop. The total reported number of dengue cases and deaths in the Philippines for 1998 were 31,297 and 493 respectively<sup>(11)</sup>.

Dengue is transmitted by the *Aedes* mosquitoes. These mosquitoes are day-biting. They especially inhabit domestic water and rain-filled containers near buildings<sup>(11)</sup>.

Dengue is especially a concern in terms of treatment and prevention. Currently there are no antiviral medications to treat dengue<sup>(12)</sup>. Also, there are no marketable vaccines for dengue prevention<sup>(13)</sup>. Dengue primarily targets school-age children<sup>(11,13,14)</sup>. Therefore, school-children were the principal recipients of a series of health education programmes in one Philippine city. In addition, school grounds and buildings as potential mosquito breeding sites were given attention in some of the Philippine campaigns<sup>(15,16)</sup>.

The prevention and control of dengue centres on the control of dengue-carrying mosquito breeding sites. Potential breeding sites include such containers as flower pots, storage containers, jars and cans inside and outside of buildings. Containers are usually divided into categories as disposable or reusable. The reusable containers need covering and regular cleaning. Health educational programmes directed towards

dengue control focus on the removal or covering of potential mosquito-breeding sites<sup>(13,16,17)</sup>.

## Methods

An eight-year old Filipino-American child who had lived the majority of his life in a dengue-endemic city of the Philippines was asked to draw and describe two games related to dengue fever. The child had prior knowledge about dengue. This study was organized during the child's first week of third grade. Parental consent and the pupil's assent was obtained prior to the initiation of the study. The case study took place in the child's home.

### Instructions for the first game

Make a game about dengue fever. The child had paper and pencil to create the game.

After approximately one-half hour the child completed the game design. After the completion of the game the child was asked to describe the game, its play and its rules.

One day later the child was asked to create another game related to dengue. Instructions for the second game were based upon gaps in details that were lacking from the child's first game responses. No feedback was given to the child after the first game and prior to the second game.

### Instructions for the second game

The child was asked to make a new game about dengue. The child was asked to make another drawing. The child could use the

design of the first game as a basis for the new game or use a different design. Prior to the second game creation the child was further instructed and asked the following:

- The game should include how mosquitoes can best be prevented.
- The game should include something about where mosquitoes grow.
- The game should include something about removing or covering containers.
- How does someone win the game?
- What are the rules of the game?
- What should a student learn from the game?
- What makes the game fun?

It took the child approximately thirty minutes to complete the second game. No time limit was given to the child for game creation.

## Results

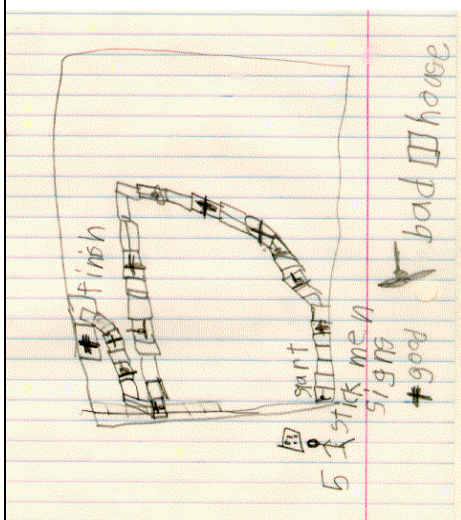
See Figure 1 for the design of the first game. The child gave verbal instructions as to the play of the game. The child stated the following:

"If you land on the mosquito space you get a mosquito token. If you land on the hospital you are cured and you take away the mosquito. The person with the least number of mosquitoes is the winner. The person with the most mosquitoes is the loser". In addition, the child indicated "if you land on a house with screens you lose a mosquito".

The components of the game included a die, and up to five players (drawn as "stick

men"). The game board included three symbols. The symbols were called "signs" by the child. The three symbols were as follows: a cross, which symbolized a hospital (indicated as good), a drawing of a mosquito (referred to as bad), and a square with a line down the middle represented a house with screens.

Figure 1. *Child's drawing of first game*

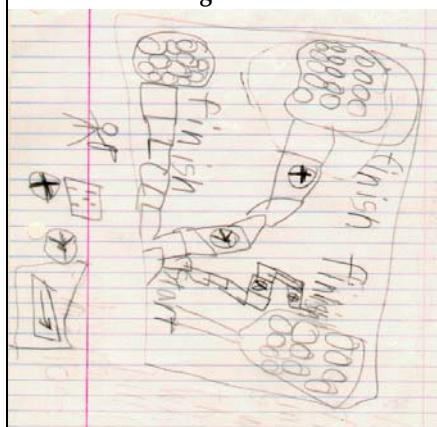


See Figure 2 for the design of the second game. The child's responses to the seven questions and instructions were as follows:

- Dengue could be prevented "by going into the bucket of mosquito eggs and killing the baby mosquito eggs or by walking on the mosquito eggs".
- With respect to where mosquitoes grow the child stated that, "they grow in water."

- The child did not indicate on the game's board design nor verbally state anything about removing or covering potential mosquito breeding-site containers.
- With respect to winning the game the child stated, "By being the first one to go to the most spots. A person needs to go to all three spots". The child later indicated that "spots" referred to the buckets with mosquito eggs. These "spots" were the circular end points on the game's board design.
- With respect to rules, the child indicated in writing, "The most ones who kills the baby mosquitoes wins. But the ones who do not kill the most baby mosquitoes loses." The child was then asked how the baby mosquitoes were killed? The child responded "by landing on it" (in reference to the buckets where the mosquito eggs were located). The above rules actually related to the winning process.

Figure 2. *Child's drawing of second game*



The child was then probed about the mechanics of movement around the game board. The child indicated that each player should start at a common location. The players would each proceed back and forth to each of the three buckets and then finally end at their designated finish point. The child indicated that points would be scored for landing on the buckets with mosquito eggs. However, points would be lost by landing on the mosquito space. Landing on the space with the mosquito indicates receiving a mosquito bite. The child further indicated that if a player “gets too much mosquito bites you also lose”. The child also indicated that landing on the spot with the hospital symbols was positive, it indicated a “cure”. The child indicated that a player will receive 10 points by landing on the mosquito eggs, lose 10 points when landing on the mosquito space (referring to receiving a mosquito bite) and gain 9 points by landing on the “cure”/or hospital space. The child further stated that landing on the space with an arrow indicated that the player could move ahead one extra space. The drawing of a figure represented a “stick man” with a spray gun. According to the child the “stick man” represented a player.

No rules about order of play or moral obligations related to play were given.

- In response to the question, what should one learn from the game the child responded, “To have fun. It helps kids know about rules so that kids won’t get mosquito bites. Like covering the buckets.”
- According to the child the game is made fun “by being the winner.”

## Discussion

The results of this study were consistent with the literature<sup>(1)</sup> in the selection of game category. Board games were the preferred form of created game for early to mid-grade level elementary school children.

Rules of the study’s invented dengue games were essentially procedural. That is, the rules dealt with the mechanics of playing the game and with a focus on winning the game. This was consistent with the literature that third graders were capable of designing procedural or winning rules. Furthermore, no winning rules were made for the games of first or second graders<sup>(1)</sup>. However, the dengue games invented in this study did not contain social or moral rules. This was in contrast to a previous study where approximately one-third of second graders had moral rules in their invented games<sup>(1)</sup>.

Parlett classified board games into five categories<sup>(18)</sup>. The two invented games could be classified as combinations of thematic and racing games. The principal theme for both games was dengue fever control. The games also included the element of a race, for example, being the first to complete the course of the game design. Both games employed the element of chance (e.g. landing on a “good” space or “bad” space on the game board was dependent upon the roll of the die) rather than skill (knowledge or abilities either in game tactics or in content about the game’s topic of dengue control). The chance element may provide a fun element for children.

Both child-invented dengue games lacked titles. Most game instructions created by the child were orally given. Thus, the game board design may have been more



important to the child than the games' instructions.

Both games revealed the child's content understanding about dengue. These content topics reflected upon the child's understanding about dengue transmission and dengue-carrying mosquito control measures. Most of the child's information was correct. However, some conceptual issues needed correction or revealed incomplete information. The following discussion will cover these issues.

### Game One discussion

The child understood that mosquitoes were in some way related to dengue fever. The exact connection to disease transmission was not specifically stated. However, the child knew that mosquitoes were in some way related to dengue. Mosquitoes were perceived as "negative". The child mentioned hospitals as a "cure". There is no actual "cure" for dengue as in an antibiotic or antiviral medication. However, fluid replacement therapy and anti-fever medication can certainly aid a person through a dengue illness<sup>(12)</sup>.

The child understood the importance of screens in the control of dengue. It appears that the child inferred that screens protected people from mosquito bites. The public health literature suggested that screens might be an important protective factor in dengue prevention<sup>(19,20)</sup>.

The child created simple but straightforward relationships in the game. They were as follows with respect to dengue: mosquitoes were negative, screens were positive and hospital also positive. This could be described as an example of the

concrete level of cognitive thinking described by Piaget<sup>(4)</sup>.

### Game Two discussion

With respect to disease transmission the child understood the importance of eliminating the mosquito eggs (earliest stage of mosquito life cycle)<sup>(13)</sup> as exemplified by game board drawings of "eggs" in buckets and comments made about "killing the baby mosquito eggs". However, the child incorrectly understood that stepping on them in a bucket could destroy mosquito eggs. The game's mechanics, however, reflected on the importance of the control of the mosquito eggs, the earliest stage of the mosquito life cycle.

Just as in the first game the child indicated that landing on the adult mosquito was negative while landing on the game board's hospital space had positive consequences. This time, the child indicated that the adult mosquito space referred to a mosquito bite; thus, demonstrating the child's view of disease transmission by mosquito bite.

The child indicated in discussion the importance of covering containers as a means of preventing dengue. Covering reusable water containers is a recommended means of controlling the dengue-carrying mosquitoes<sup>(17)</sup>. However, the child in the current dengue game study did not integrate this concept into the game. Another concept of dengue-related mosquito control that was missing was that of the removal of disposable mosquito breeding site containers<sup>(17)</sup>.

An incomplete understanding of the mosquito life cycle may result in the stress on less effective control measures, such as an

emphasis on adult mosquito control through insecticide spraying. Winch et al., in their study of beliefs related to dengue control in Mexico, suggested that “people do nothing to control dengue because they do not understand the life cycle of the mosquito”<sup>(21)</sup>.

Previous studies of both a combined parents-teachers group and also students in the Philippines yielded the rating of the importance of controlling adult mosquitoes over mosquito larva control<sup>(15,16)</sup>. It is therefore interesting to observe that the child drew an insecticide spray gun in the hand of the figure representing the player. Insecticide spray guns or spray cans are used for adult mosquito control. This appeared to be in conflict with the child’s earlier statement about “killing the baby mosquito eggs” as a key method to prevent dengue. The drawing of the player with the insecticide “spray gun” in hand is consistent with the beliefs about dengue-related mosquito control held by other age groups<sup>(15,16)</sup>.

A major manufacturer of insecticide spray has begun a dengue information campaign in Philippine elementary schools<sup>(22)</sup>. Without the emphasis on mosquito larval control, such a dengue campaign will be incomplete. In actuality, an over-emphasis on insecticide use and other commercial protective measures may actually reinforce the notion among elementary school-age children that removal or covering mosquito breeding sites was subordinate to other dengue control measures.

Dengue fever and dengue haemorrhagic fever are important child health concerns for children in the

Philippines and other dengue-endemic countries in the tropical zones<sup>(7,11,14)</sup>. Dengue is an important theme for children’s games in dengue-affected areas. However, children can learn from game designs from other cultures and countries<sup>(23)</sup>. It may be valuable for children to learn about themes affecting people in other countries and cultures through the vehicle of educational games. Dengue may be an important theme for children living outside the dengue zone to learn about as well.

## **Recommendations and conclusion**

These dengue game exercises may be conducted as home or school activities. Also, they may be conducted individually or in groups. The children’s play of these prototypes may result in suggestions for game refinement. These refinements may result in more workable games as educational tools.

The dengue game invention exercises for a child in this study may have multiple uses. These activities may be diagnostic of a child’s understanding of a topical knowledge (in this case dengue), provide a child-centred educational tool at the appropriate educational age level, measure a child’s cognitive level, and provide potential formative research information for dengue prevention and control programmes.

## **Acknowledgements**

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## Dengue Prevention and Control

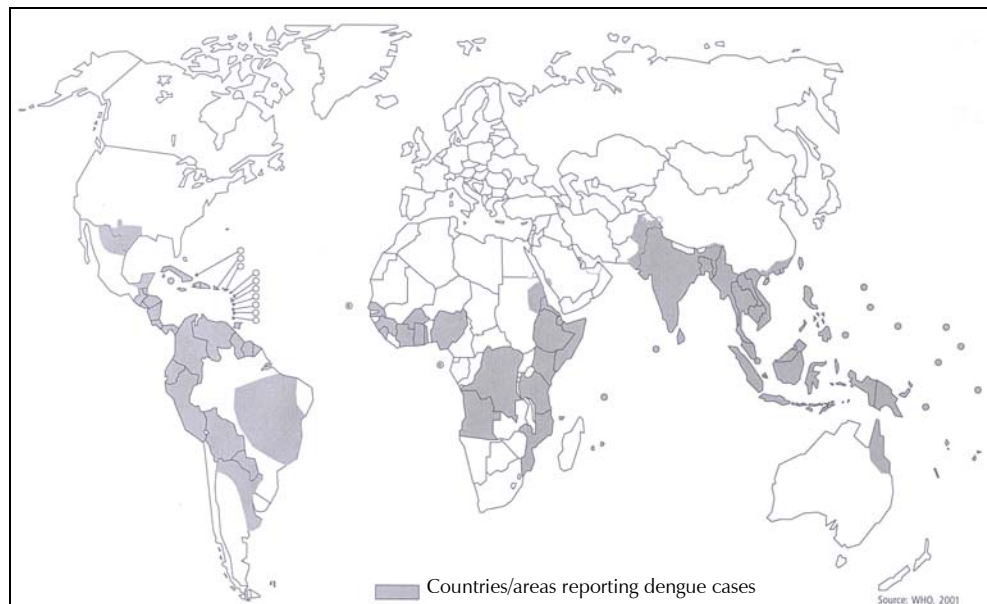
*Weekly Epidemiological Record 2002, 76(29): 217-218*

Dengue is the most common and widespread arthropod-borne viral infection in the world. There are 4 distinct virus serotypes, each capable of producing a wide spectrum of signs and symptoms that characterize dengue fever, ranging from sub clinical infection, to a debilitating but self-limiting illness with symptoms resembling influenza, to severe disease known as dengue haemorrhagic fever. Without proper

hospital care, the latter can lead to clinical shock and death in less than 24 hours

The geographical spread, incidence and severity of dengue fever and dengue haemorrhagic fever are increasing in the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific Regions (Figure).

Figure: **Dengue, 2001**



Before 1970, only 9 countries had experienced dengue haemorrhagic fever. Since then, the number has increased more than fourfold and continues to rise. Some 2.5-3 billion people live in areas where dengue viruses can be transmitted. A pandemic in 1998, in which 1.2 million cases of dengue fever and dengue haemorrhagic fever were reported from 56 countries, was unprecedented. Preliminary data for 2001 indicate a situation of comparable magnitude. However, only a small proportion of cases are reported to WHO; it is estimated that each year 50 million infections occur, with 500,000 cases of dengue haemorrhagic fever and at least 12,000 deaths, mainly among children, although fatalities could be twice as high.

Without proper clinical management, case-fatality rates for dengue haemorrhagic fever can exceed 20%. However, with intensive supportive therapy, rates can be reduced to less than 1%.

The resurgence of epidemic dengue fever and the emergence of dengue haemorrhagic fever as major public health problems are rooted in the demographic trends and socioeconomic policies of the 20<sup>th</sup> century. During the past five decades, the population of the world has more than doubled, with the most rapid acceleration taking place in developing countries in the tropics and subtropics where dengue viruses are spread by mosquitoes. Several factors have combined to produce epidemiological conditions that highly favour viral transmission by the main mosquito vector, *Aedes aegypti*: population growth, rural-urban migration, the inadequacy of basic urban infrastructure (e.g. unreliable water supply, which may lead householders to

collect and store water close to homes) and the huge increase in volume of solid waste resulting from the new habits of consumers, for example, discarded plastic containers and other abandoned items which provide larval habitats in urban areas. The species thrives in intimate association with humans and is also the vector of the virus of urban yellow fever, a vaccine-preventable disease. A secondary vector of dengue virus, *Ae. albopictus*, which until the late 1970s was geographically limited to parts of Asia, has now become established in Africa, the Americas and Europe. Geographical expansion of this mosquito has been aided particularly by international commercial trade in used tyres which, with accumulated rainwater, are attractive habitats for egg-laying females of the species. Its role in the transmission of dengue and potentially also of yellow fever and other arthropod-borne viruses in these new epidemiological settings remains to be determined. The magnitude of the public health problem will continue to grow unless more effective measures are taken to reduce viral transmission.

In many countries, health-sector reform poses new challenges for programme delivery, including decentralization and issues of selection, purchase, procurement, use and monitoring of insecticide application. Moreover, few new cost-effective chemical pesticides suitable for public health use have been developed in recent years. This problem is particularly acute with regard to larvicides suitable for use in stored water for domestic consumption.

Although research on dengue vaccines for public health use is in process, currently the only method for the prevention and control of the disease is vector control.

The global strategy enunciated in 1995<sup>1</sup> recommended the application of integrated vector control measures, with community and intersectoral participation. An informal WHO consultation on strengthening implementation of the global strategy for prevention and control of dengue fever/dengue haemorrhagic fever (Geneva, 18-20 October 1999), the subsequent inclusion of dengue in the disease portfolio of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases in June 2000, and advances in regional strategy formulation in the Americas, South-East Asia and the Western Pacific during the 1990s, have facilitated identification of the following four main priorities:

- (1) Strengthening epidemiological surveillance for planning and response, including entomological surveillance and the monitoring of key human behaviours (such as inappropriate disposal of discarded household items) that contribute to the availability of mosquito larval habitats. Epidemiological surveillance includes the introduction of DengueNet, a global surveillance system for dengue fever on the Internet. This network includes a database which will be continually updated and which will allow remote data entry in order to

provide a more comprehensive and current global picture.

- (2) Reducing the disease burden through: accelerated training and the adoption of WHO standard clinical management guidelines for dengue haemorrhagic fever; improving emergency preparedness and response, and strengthening of national vector-control programmes.
- (3) Promoting behavioural change through the development and implementation of a package of tools, approaches and guidelines for sustainable prevention and control of vectors at individual, household, community, institutional and political levels. The approaches will also foster intra- and intersectoral partnerships for programme implementation.
- (4) Accelerating the research programme, with emphasis on mechanisms of pathogenesis, transmission dynamics, vaccine development, validation and improvement of existing or new vector-control methods and their application, partnership building, and formulation of guidelines for research in these strategic areas.

Given the worsening epidemiological trends, there is evident need to renew or intensify efforts to reduce the public health and economic burdens associated with this epidemic disease. In order to achieve this, the following will be required: the development, application and evaluation of

<sup>1</sup> Report of the consultation on key issues in dengue vector control toward the operationalization of a global strategy, WHO, Geneva, 6-10 June 1995, document CTD/FIL(DEN)/IC/96.1). Can be obtained on request from the CDS Information Resource Centre, World Health Organization, 1211 Geneva 27, Switzerland; fax: +41 22 791 42 85; email: cdsdoc@who.int)

new and improved tools and strategies for the prevention and control of dengue fever and dengue haemorrhagic fever; increased commitment and additional human and other resources for improved and sustainable prevention and control efforts; building and strengthening the capacity of health systems

for dengue surveillance, laboratory diagnosis and disease management, and active intersectoral partnerships involving international, regional, national and local agencies and nongovernmental organizations.

# **DengueNet<sup>1</sup> – WHO's Internet-based System for Global Surveillance of Dengue Fever and Dengue Haemorrhagic Fever (Dengue/DHF)**

**<http://www.who.int/denguenet>**

*Weekly Epidemiological Record 2002, 77(36): 301-304*

## **Dengue/DHF – global public health burden**

The geographical spread of both the mosquito vectors and the viruses over the past 25 years has led to the global resurgence of epidemic dengue fever/dengue haemorrhagic fever (dengue/DHF), with the development of hyperendemicity in most urban centres in the tropics. Globally, 2.5 billion people live in areas where dengue viruses can be transmitted. Before 1970, only nine countries had experienced epidemic DHF; now the number has increased more than fourfold and continues to rise. In an unprecedented pandemic in 1998, 1.2 million cases of dengue fever and DHF were reported to WHO from 56 countries. Data for 2001-2002 indicate a comparable situation. It is estimated that 50 million dengue infections occur each year, with 500,000 cases of DHF and at least 12,000 deaths, mainly among children. Only a small proportion of cases are reported to WHO. The challenge for national and international

health agencies is to reverse the trend of increased epidemic dengue activity and increased DHF incidence.

## **Rationale for global surveillance of dengue/DHF**

Epidemiological and laboratory-based surveillance is required to monitor and guide dengue/DHF prevention and control programmes, regardless of whether control takes the form of mosquito control or possible vaccination if an effective and safe vaccine becomes available. The surveillance system should monitor dengue virus to show, at any point in time, where dengue transmission is occurring, what serotypes are involved, and what type of illness is associated with those serotypes. Case reports should be transmitted from the local level to the state/provincial and then national level, and from there to WHO for international reporting and use. However, the reporting of dengue/DHF is not standardized. Epidemiological and laboratory data are often collected by different institutions and

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<sup>1</sup> DengueNet has been developed in collaboration with the WHO Collaborating Centre for Electronic Disease Surveillance at the Institut national de la Santé et de la Recherche médicale, INSERM Paris, France



reported in different formats, resulting in delay and comparability problems at regional and international levels. To address these problems WHO has created DengueNet.

The DengueNet system responds to the WHO resolution on dengue fever/DHF prevention and control adopted at the 55<sup>th</sup> World Health Assembly in May 2002, asking Member States “to build and strengthen the capacity of health systems for surveillance, prevention, control and management of dengue and DHF”, and emphasizing the critical importance of strengthening laboratory diagnosis in affected countries. It is in line with the principles developed by PAHO for epidemiological and laboratory surveillance of dengue/DHF in the Americas, as outlined in resolution CD43.R4 and working document CD43/12, adopted by the PAHO Directive Council in September 2001.

## **DengueNet – WHO's Internet-based system for global surveillance of dengue/DHF**

WHO has created DengueNet as a central data management system to:

- (1) collect and analyse standardized epidemiological and virological data in a timely manner, and to present epidemiological trends, as soon as new data are entered;
- (2) display in real-time important indicators such as incidence data, case-fatality rates (CFR) for DHF, frequency and distribution of dengue and DHF cases, number of

deaths, and distribution of circulating dengue virus serotypes, and

- (3) provide both historical and real-time data.

The main features of this Internet-based surveillance tool are:

- (1) password-protected capability for remote data entry by all DengueNet partners worldwide, with data updated on a real-time basis;
- (2) inclusion of the state/province subdivisions of the countries for which data will be entered and indicators (such as incidence) calculated;
- (3) dynamic query facility with analysis and presentation of data in graphic, tabular, map and free-text formats;
- (4) use of GIS tools to provide a real-time map of the epidemiological situation;
- (5) links to the dengue web pages of WHO offices, countries, collaborating centers, and research and medical institutions working worldwide on dengue/DHF prevention and control;
- (6) an up-to-date directory of national and international partners in the DengueNet network;

At present, global dengue statistics from 1955 to 2001 can be accessed on DengueNet. As countries begin entering data into DengueNet, real-time updates of standardized epidemiological and virological

data will become available, when DengueNet is fully implemented, public health authorities and the general public will have immediate access to epidemiological data on dengue, DHF cases and deaths, based on standardized case definitions, and virological data on the circulating dengue virus serotypes 1, 2, 3 and 4 that have been entered into the DengueNet database via the Internet directly by the national health officials.

DengueNet will provide national and international public health authorities with epidemiological and virological information, by place and time, to guide public health prevention and control actions. Monitoring virus transmission and circulating serotypes by place and time in the inter-epidemic periods will provide early warning of dengue activity in neighbouring states/countries and help in the planning of prevention or control strategies. This is particularly important in the Region of the Americas, which is characterized by unstable dengue epidemic activity with emerging DHF cases.

The system also provides CFR information by place and time, and this can be used effectively to target training to countries and regions that need to improve hospital-based DHF case management to reduce CFR. This is particularly important in South-East Asia, where all four dengue viruses are endemic and DHF cases occur year after year; CFR are used to monitor progress in hospital case management and public education campaigns.

In addition, DengueNet contains valuable historical and current data that may be useful for public health researchers to support their research and for national and international agencies for advocacy purposes.

A key objective is to ensure that data of the highest possible quality are reported in a timely manner to DengueNet. This necessitates standards for surveillance, laboratory procedures and quality control supported by a strong partnership between the network partners involved, including national programmes, WHO collaborating centres and WHO country, regional and global levels.

## **DengueNet implementation**

The first meeting on DengueNet implementation in the Americas was held on 9-11 July 2002 in Puerto Rico.<sup>2</sup> The specific objective was to launch pilot testing by building on the existing reporting systems and the network of dengue laboratories in the Americas.

## **Purpose and objective**

Forty participants<sup>3</sup> (surveillance epidemiologists and laboratory specialists) from 15 countries participated in this first meeting on implementation of DengueNet. The overall objective was to describe and demonstrate DengueNet to prospective users and to

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2 This meeting was organized by the WHO Department of Communicable Disease Surveillance and Response, Global Alert and Response, jointly with the PAHO Division of Communicable Disease Prevention and Control and the WHO Collaborating Centre for Dengue Reference and Research at the Dengue Branch of the Division of Vector-Borne Infectious Diseases, US Centers for Disease Control and Prevention.

3 National programmes from Brazil, el Salvador, French Guyana, Guatemala, Mexico, Nicaragua, Puerto Rico, Venezuela; CAREC (Trinidad and Tobago), the subregional surveillance network for 20 island countries in the Americas; WHO Collaborating Centres and research institutions in Argentina, Brazil, Canada, Cuba, United States; participants from Indonesia, Thailand, and Viet Nam who will assist WHO in organizing a DengueNet meeting in 2003 for high-burden countries in South-East Asia and the Western Pacific; WHO/HQ, PAHO and WHO/PAHO Country Offices in Brazil and Nicaragua.

develop a framework for DengueNet implementation with emphasis on quality of data entered and the active participation of national programmes. Technical discussion focused on (1) the challenge of and need for global epidemiological and laboratory surveillance of dengue and DHF; (2) national epidemiology and laboratory capacities in participating countries in the Americas; (3) presentation of DengueNet and a "hands-on" session with the Internet site. Two working groups were convened. The first defined the epidemiological data and reporting requirements for DengueNet, modifications needed to its present format, identification of countries for pilot testing, and the roles and responsibilities of national and international partners. The second group reviewed laboratory standards and quality control issues for dengue serological diagnosis and virus isolation, building on the recommendations of two previous WHO meetings on dengue laboratories in the Americas.<sup>4</sup>

## Meeting outcomes

The first meeting marked the start of the phased implementation of DengueNet starting with the Americas in 2002 and expanding to South-East Asian and Western Pacific Regions in 2003. There was very active participation of participants from national programmes and laboratories, WHO collaborating centres and WHO/HQ, PAHO and country offices. The key outcomes of these discussions are summarized below.

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<sup>4</sup> The 2 WHO meetings were held in Cincinnati (USA), in 1994 and in Rio de Janeiro (Brazil), in 1996.

## Data collection

### *Epidemiological data*

Countries will provide these data by epidemiological week at state/department level for the large countries and at the island level for island countries. The data reported in DengueNet will include the clinical categories of dengue fever, DHF, both suspected and confirmed cases, and only confirmed dengue deaths.

Case-fatality rate will be calculated as follows:

$$CFR = \frac{\text{confirmed deaths}}{\text{confirmed cases of DHF}}$$

### **Virus serotype data – all available**

In the pilot test, these data will be provided for the entire country and be displayed in DengueNet as the cumulative number of isolations of each serotype in the country from 1 January.

DengueNet will calculate the number of isolations of each serotype as a percentage of total isolations of all four serotypes in the country from 1 January, as, for example:

$$\% \text{ Den-1} = \frac{[\text{Den-1}]}{[\text{Den-1} + \text{Den-2} + \text{Den-3} + \text{Den-4}]} \times 100$$

### *General considerations*

Data will be provided only by the central level of each country (one source of data per country). DengueNet will link to the country web pages for additional information. The data entered during the pilot testing period will include a disclaimer stating that the

system is being tested and that the data for this period are provisional.

### **Roles and responsibilities of the partners in this network**

Countries will collect, validate and provide epidemiological and laboratory data, and will designate the participating centres. The WHO collaborating centres will continue to provide laboratory support, proficiency panels and training to national laboratories. PAHO will support the country implementation activities, and WHO/HQ will maintain and moderate the DengueNet web site. Both PAHO and WHO/HQ will seek financial support for dengue surveillance activities.

### **Country participation**

A major outcome of the meeting was that all the representatives of countries in the Americas expressed interest in participating in the DengueNet pilot test, and the representatives of South-East Asian countries indicated interest in the system being expanded to include their Region. The participants will approach their country authorities to obtain official authorization to participate in DengueNet. WHO country representatives will support the participants in presenting the DengueNet proposal to the national authorities. The pilot testing of DengueNet in the Americas will be conducted over a period of 3-6 months. The lessons learnt will be built into the implementation framework for high-burden countries in the South-East Asian and Western Pacific Regions in 2003.

# Revision of International Health Regulations: Progress Report, May 2002

*Weekly Epidemiological Record*\* 2002, 77(19): 157-160

The stated purpose of the International Health Regulations (IHR) is "to ensure the maximum security against the international spread of diseases with a minimum interference with world traffic"<sup>1</sup>. The current regulations consist of provisions for the reporting to WHO of cases of three diseases (cholera, plague and yellow fever), and a series of articles setting out measures to be implemented at points of arrival and departure (ports, airports and frontier posts) and in international conveyances (ships, aircraft, etc.). In 1995, the World Health Assembly adopted resolution WHA48.7 on "Revision and updating of the International Health Regulations" requesting that the IHR be revised to take more effective account of the threat posed by the international spread of new and re-emerging diseases. Since the 1995 resolution, regular updates on progress of the revision project have been published in the *Weekly epidemiological record*.<sup>2</sup>

## Global health security: epidemic alert and response

In 2001, the World Health Assembly adopted resolution WHA54.14 on "Global

\* <http://www.who.int/wer>

health security: epidemic alert and response" in which the revision of the IHR is explicitly linked to WHO's activities to support its Member States in identifying, verifying and responding to "health emergencies of international concern". In addition, support was expressed for two key elements of the proposed IHR revision: the development of criteria to define what constitutes a public health emergency of international concern for notification under the revised regulations and the identification by all WHO Member States of national focal points to work with the IHR project in WHO.

The WHO strategy for Global health security: epidemic alert and response (Figure) has three main components: (i) specific programmes for the prevention and control of known epidemic threats such as influenza, meningococcal disease or cholera; (ii) the detection and response to health emergencies resulting from unexpected circumstances or unknown aetiologies, and (iii) the improving of preparedness through the strengthening of national infrastructures for disease surveillance and response. These three types of activity are fundamentally

<sup>1</sup> International health regulations (1969) adopted by the Twenty-second World Health Assembly in 1969 and amended by the Twenty-sixth World Health Assembly in 1973 and the Thirty-fourth World Health Assembly in 1981, 3<sup>rd</sup> annotated ed. Geneva, World Health Organization, 1983

<sup>2</sup> See No. 31, 1996, pp. 233-234; No. 3, 1997, pp. 9-11; No. 29, 1997, pp. 213-215; No. 4, 1998, pp. 17-19; No. 31, 1998, pp. 233-237; No. 4, 1999, pp. 25-27; No. 30, 1999, pp. 252-253; No. 4, 2000, pp. 35-36; No. 29, pp. 234-236; No. 8, 2001, pp. 61-63.

underpinned by the partnerships that WHO has built up with international agencies and a wide variety of institutions around the globe. The revised IHR will provide the necessary framework within WHO's global health mandate to carry out these activities. The implementation of the WHO strategy for Global health security: epidemic alert and response will link the IHR with activities at global and national levels, requiring significant commitment from all countries. From this perspective, the formal adoption of the IHR by the Health Assembly will provide the necessary and visible commitment from WHO's Member States.

## Public health emergencies of international concern

It has always been central to the IHR revision process that the requirement to notify WHO has to be broadened in scope from the present three diseases listed in the regulations. The pilot study undertaken in 1998 demonstrated that replacement of the listed diseases by a series of syndromes was not a feasible approach from the regulatory viewpoint. WHO has since worked with the Swedish Institute of Infectious Diseases to define the type of health-related events that would ideally be notified under the revised regulations. This partnership has resulted in the development of a limited number of criteria that can be used to identify public health emergencies of international concern. A tool, based on these criteria, for use at global and national level will be tested in the next six months prior to incorporation into the revised draft regulations. The tool will assist the decision on the revised regulations to notify or not by answering four key questions: Is the event serious? Is the event

unexpected? Is it likely that there will be international spread? And is it likely that the event will result in international restrictions to travel or trade?

In addition to defining the health emergencies to be notified, it is proposed that the revised IHR define the capacities that a national disease surveillance system will require in order for such emergencies to be detected, evaluated and responded to in a timely manner. Such capacities already exist in some Member States, but it is recognized that many national systems will not be able to achieve such a level of functionality by the time the revised regulations are planned to come before the Health Assembly in 2004. For these countries, a clear statement of what the IHR requires from a surveillance system will be a useful target to aim for in strengthening national disease surveillance and response systems and achieving a globally agreed minimum standard.

The scope of the revised IHR will be increased to take account of information coming from sources in addition to official notification by Member States. The established WHO outbreak verification system<sup>3</sup> already receives information about outbreak events from a variety of sources.

Under the revised IHR, WHO will undertake to support Member States with technical assistance at their request in taking the appropriate measures to investigate, control and contain the emergencies notified. Through the Global Outbreak Alert and Response Network, established in April

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3 Grein TW. Et al. Rumours of disease in the global village: outbreak verification. *Emerging infectious diseases*, 2000, 6: 97-102

2000, WHO can secure the support required from among over 100 network partners able to provide the international community with highly qualified staff and special technical supplies. Since early 2000, WHO and the Network have launched effective international responses to outbreaks in more than 12 countries.

The revised IHR will provide for the recommendation by WHO of time-limited measures, based on WHO's assessment of the risk associated with any public health emergency notified.

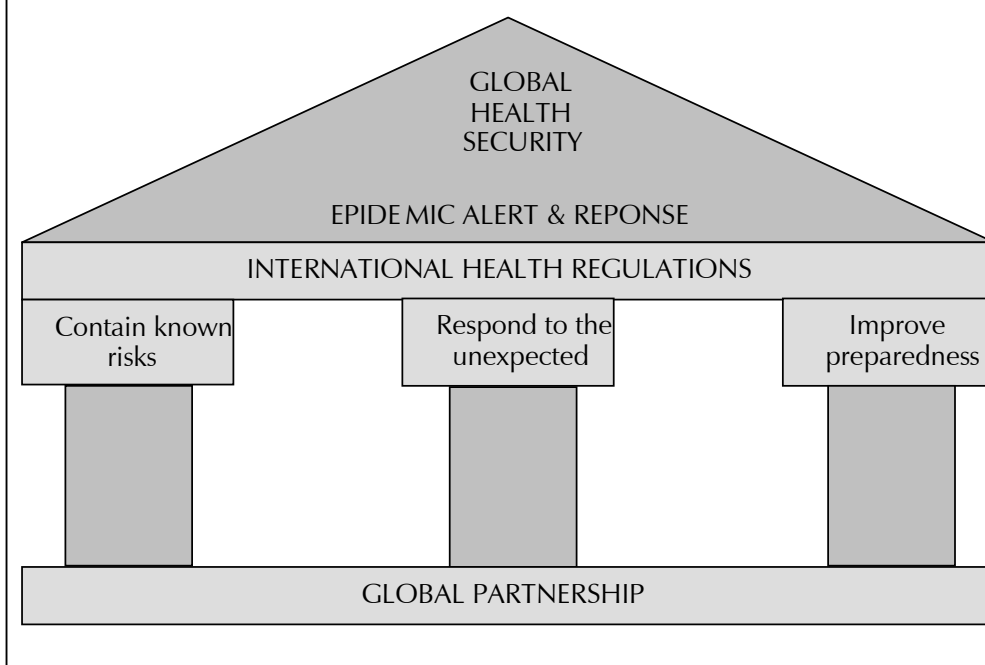
## Routine preventive measures

The routine preventive measures already provided in the current IHR will be updated

to take account of changes in the nature and extent of international travel and commerce as well as advances in the technology of the control of environmental disease hazards.

The IHR will continue to make direct reference to WHO guidelines that provide technical recommendations about how to achieve the requirements of the IHR. The *Guide to ship sanitation* and the *Guide to hygiene and sanitation in aviation* are both being revised and updated by the WHO Programme on Sustainable Development and Healthy Environments. Two new guides will be developed for the revised IHR; a guide to early warning systems in disease surveillance and an operational guide to facilitate the application of the revised IHR.

Figure: *Illustration of the WHO strategy for global health security*



## **IHR focal points**

The revised regulations will include the obligation for Member States to collaborate with WHO, through nominated national IHR focal points, to verify the content of reports obtained from non-official sources where the events described may meet the criteria for notification to WHO.

National IHR focal points will become a key means of communication between the Organization and its Member States for both the IHR revision process and the implementation of the revised regulations. After adoption of the regulations, the focal points will be the means through which a dialogue can be held with WHO regarding the notification of any health emergencies under the regulations. The focal point will also be the channel through which recommended measures to contain a public health emergency of international concern will be disseminated to Member States.

## **Revision process**

The IHR were created for the Member States of WHO and can only be adopted and

implemented by Member States: it is therefore crucial to the revision project that countries participate in the process at every stage. The project team is seeking to facilitate this involvement through the identification of national focal points, through individual country visits where these are requested, through active participation at global, regional and sub-regional meetings on issues relevant to the IHR and through inviting national representation at expert meetings held to develop the revised regulations. The first (non-regulatory) draft of the revised regulations will be shared with Member States at the end of 2002, at which time meetings are planned for all WHO regions with the objective of achieving wide consensus on the nature of the public health provisions to be included in the regulatory text. It is planned that the first regulatory draft will be sent out for discussion early in 2003 with a final version available by October 2003 in order that it can be put forward for adoption by the Health Assembly in 2004.



# Vaccines and Biologicals Recommendations from the Strategic Advisory Group of Experts

*Weekly Epidemiological Record 2002, 77(37): 305-311*

## Yellow fever

There was concern regarding the resurgence of yellow fever (YF) over the past 20 years in many countries, especially those in West Africa. This is very distressing in view of the availability of a safe and inexpensive vaccine for more than 60 years. This disease can be readily controlled using currently available vaccines.

Noting that routine yellow fever vaccination is endorsed by the World Health Assembly (WHA), the Strategic Advisory Group of Experts (SAGE) strongly endorses the additional strategy of targeting "high-risk" districts or both routine infant immunization and conducting one-off "catch-up" campaigns. These campaigns target persons 9 months of age and above for YF vaccination. It is helpful to have a well-functioning routine immunization system before conducting a preventive mass campaign. However, it is not necessary to wait for YF vaccine to be included in the routine immunization system before conducting such a campaign (so long as there is not a long interval between the two activities). Indeed, a campaign can be used as an opportunity to "jump-start" the inclusion of YF vaccine in routine immunization services.

SAGE makes the following recommendations:

- (1) WHO/UNICEF should jointly develop a 5-year strategic plan for yellow fever control, including a detailed forecast of vaccine demand and supply and the creation of an emergency YF stockpile.
- (2) While YF vaccine is generally considered to be safe, recent reports of serious adverse events following YF vaccination are of concern. The efforts to enhance surveillance for and investigation of serious adverse events following YF vaccination, initiated by WHO, should continue. An evaluation of this work should be presented to SAGE in 2003.

## Yellow-fever Vaccinating Centres

*Weekly Epidemiological Record 2002, 77(8): 61*

The designation of yellow-fever vaccinating centres is the responsibility of national health administrations according to the requirements of the International Health Regulations, Article 66, paragraph 4, which states: "The yellow-fever vaccine used must be approved by the Organization, and the vaccinating centre must have been designated by the health administration for the territory in which it is situated...".

In recent years the number of centres designated has increased considerably in many countries and it is no longer possible to maintain an up-to-date list of all centres which would be available continuously to all health administrations. For this reason, it was decided in 1997 to cease publication of the names of these centres. It is therefore no longer necessary for health administrations to inform WHO of the designation of new yellow-fever vaccinating centres. Anyone wishing to obtain information on such centres should address questions directly to the national health administration of the country concerned.

The list of approved yellow-fever vaccine producers is published annually in *International Travel and Health*.

For easy reference the list as of 1 January 2002 is as follows:

Aventis Pasteur  
58, avenue Leclerc  
BP 7046  
69348 Lyon Cedex 07  
France

BioManguinhos  
Av Brasil 4365 – Manguinhos  
21045-900 rio de Janeiro/RJ  
Brazil

Institut Pasteur Dakar  
BP 220  
36, avenue Pasteur  
Dakar  
Senegal

Celltech Group plc (formerly Medeva, U.K.)  
Evans House, Regent Park  
Kingston Road  
Leatherhead LT22 7PQ  
United Kingdom

Any updates are given at the following web address:

[http://www.who.int/vaccines-access/vaccines/Vaccine\\_Quality/UN\\_Pre-qualified/unyf\\_producers.html](http://www.who.int/vaccines-access/vaccines/Vaccine_Quality/UN_Pre-qualified/unyf_producers.html)

# Dengue Prevention and Control

*Report by the Secretariat of Fifty-fifth World Health Assembly, AA/19,  
Provisional Agenda Item 13.14 dated 4 March 2002*

Dengue is the most common and widespread arthropod-borne viral infection in the world. There are four distinct virus serotypes, each capable of producing a wide spectrum of signs and symptoms that characterize dengue fever, ranging from subclinical infection, to a debilitating but self-limiting illness with symptoms resembling influenza, to severe disease known as dengue haemorrhagic fever. Without proper hospital care, the latter can lead to clinical shock and death in less than 24 hours.

The geographical spread, incidence and severity of dengue fever and dengue haemorrhagic fever are increasing in the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific. Before 1970, only nine countries had experienced dengue haemorrhagic fever. Since then, the number has increased more than fourfold and continues to rise. Some 2500 million to 3000 million people live in areas where dengue viruses can be transmitted. A pandemic in 1998, in which 1.2 million cases of dengue fever and dengue haemorrhagic fever were reported from 56 countries, was unprecedented. Preliminary data for 2001 indicate a situation of comparable magnitude. However, only a small proportion of cases are reported to WHO; it is estimated that

each year 50 million infections occur, with 500 000 cases of dengue haemorrhagic fever and at least 12 000 deaths, mainly among children, although fatalities could be twice as high.

Without proper clinical management, case-fatality rates for dengue haemorrhagic fever can exceed 20%. However, with intensive supportive therapy, rates can be reduced to less than 1%.

The resurgence of epidemic dengue fever and the emergence of dengue haemorrhagic fever as major public health problems are rooted in the demographic trends and socioeconomic policies of the twentieth century. During the past five decades, the population of the world has more than doubled, with the most rapid acceleration taking place in developing countries in the tropics and subtropics where the mosquito-borne dengue viruses are spread. Several factors have combined to produce epidemiological conditions that highly favour viral transmission by the main mosquito vector, *Aedes aegypti*: population growth, rural-urban migration, the inadequacy of basic urban infrastructure (e.g. unreliable water supply, which may lead householders to collect and store water close to homes) and the huge increase in volume of solid waste resulting from the new habits of consumers, for example, discarded

plastic containers and other abandoned items which provide larval habitats in urban areas. The species thrives in intimate association with humans and is also the vector of the virus of urban yellow fever, a vaccine-preventable disease. A secondary vector of dengue virus, *A. albopictus*, which until the late 1970s was geographically limited to parts of Asia, has now become established in Africa, the Americas and Europe. Geographical expansion of this mosquito has been aided particularly by international commercial trade in used tyres which, with accumulated rainwater, are attractive habitats for egg-laying females of the species. Its role in the transmission of dengue and potentially also of yellow fever and other arthropod-borne viruses in these new epidemiological settings remains to be determined. The magnitude of the public health problem will continue to grow unless more effective measures are taken to reduce viral transmission.

In many countries, health-sector reform poses new challenges for programme delivery, including decentralization and issues of selection, purchase, procurement, use and monitoring of insecticide application. Moreover, few new cost-effective chemical pesticides suitable for public health use have been developed in recent years. This problem is particularly acute with regard to larvicides suitable for use in stored water for domestic consumption.

Although research on dengue vaccines for public health use is in process, currently the only method for the prevention and control of the disease is vector control. The global strategy enunciated in 1995<sup>1</sup>

recommended the application of integrated vector-control measures, with community and intersectoral participation. An informal WHO consultation on strengthening implementation of the global strategy for prevention and control of dengue fever/dengue haemorrhagic fever (Geneva, 18-20 October 1999), the subsequent inclusion of dengue in the disease portfolio of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases in June 2000, and advances in regional strategy formulation in the Americas, South-East Asia and the Western Pacific during the 1990s have facilitated identification of the following four main priorities:

- (1) strengthening epidemiological surveillance for planning and response, including entomological surveillance and the monitoring of key human behaviours (such as inappropriate disposal of discarded household items) that contribute to the availability of mosquito larval habitats. Epidemiological surveillance includes the introduction of DengueNet, a global surveillance system for dengue fever on the Internet. This network includes a database which will be continually updated and which will allow remote data entry in order to provide a more comprehensive and current global picture;
- (2) reducing the disease burden through: accelerated training and the adoption of WHO standard clinical management guidelines for dengue haemorrhagic fever;

- improving emergency preparedness and response; and strengthening of national vector-control programmes;
- (3) promoting behavioural change through the development and implementation of a package of tools, approaches and guidelines for sustainable prevention and control of vectors at individual, household, community, institutional and political levels. The approaches will also foster intra and inter-sectoral partnerships for programme implementation;
  - (4) accelerating the research programme, with emphasis on mechanisms of pathogenesis, transmission dynamics, vaccine development, validation and improvement of existing or new vector-control methods and their application, partnership building, and formulation of guidelines for research in these strategic areas.

Given the worsening epidemiological trends, there is evident need to renew or intensify efforts to reduce the public health and economic burdens associated with this epidemic disease. In order to achieve this, the following will be required: the development, application and evaluation of new and improved tools and strategies for the prevention and control of dengue fever and dengue haemorrhagic fever; increased commitment and additional human and other resources for improved and sustainable prevention and control efforts; building and strengthening the capacity of health systems for dengue surveillance, laboratory diagnosis and disease management, and active inter-sectoral partnerships involving international, regional, national and local agencies and nongovernmental organizations.

### **Action by the Health Assembly**

The Health Assembly is invited to consider adoption of the resolution contained in resolution EB109.R4.

# Dengue Fever and Dengue Haemorrhagic Fever

## Prevention and Control

*Resolution of Fifty-fifth World Health Assembly, WHA55.17,  
Agenda Item 13.14 dated 18 May 2002*

The Fifty-fifth World Health Assembly,

Recalling resolution WHA46.31 and resolutions CD31.R26, CD33.R19 and CD43.R4 of the Directing Council of the Pan American Health Organization on dengue prevention and control;

Concerned that an estimated 50 million dengue infections occur annually and that the geographical spread, incidence, and severity of dengue fever and dengue haemorrhagic fever are increasing in the tropics;

Recognizing the growing burden of disease, particularly among children, and the social and economic impact of dengue epidemics;

Acknowledging the progress made in reducing the case-fatality rates of dengue haemorrhagic fever in some countries;

Appreciating that significant advances have been made in the development of dengue vaccines, although they are not yet available for public health use;

Recognizing that prevention or reduction of dengue viral transmission entirely depends on control of the mosquito vector *Aedes aegypti* and, to a lesser extent, *A. albopictus* and other secondary vector species;

Aware that dengue vector-control programmes have had considerable success in the past, but that sustained suppression of vector populations today largely depends on commitment of governments and community participation in both planning and intervention strategies and implementation of control measures to prevent breeding of *A. aegypti*;

Further acknowledging that, at the International Conference on Dengue and Dengue Haemorrhagic Fever (Chiang Mai, Thailand, 20-24 November 2000), more than 700 public health specialists from 41 countries recommended that all countries at risk of dengue viral transmission should develop and implement sustainable prevention and control programmes,

URGES Member States:

- (1) to advocate increased commitment and allocation of additional human and other resources for improved and sustained prevention and control efforts and for strengthened research;
- (2) to build and strengthen the capacity of health systems for management, surveillance, prevention, control and management of dengue fever and dengue haemorrhagic fever;

- (3) to strengthen the capacity of diagnostic laboratories, taking into account the fundamental importance of laboratory diagnosis to confirm etiology and to strengthen clinical and epidemiological surveillance for dengue fever and dengue haemorrhagic fever;
- (4) to promote active intersectoral partnerships involving international, regional, national and local agencies, nongovernmental organizations, foundations, the private sector, community and civic organizations;
- (5) to pursue, encourage and support the development, application, evaluation and research of new and improved tools and strategies for prevention and control of dengue fever and dengue haemorrhagic fever;
- (6) to strengthen health measures at borders for vector control and opportunities for diagnosis and treatment in order to optimize regional resources;

URGES other specialized agencies, bodies and programmes of the United Nations system, bilateral development agencies, nongovernmental organizations and other concerned groups to increase their cooperation in dengue fever prevention and control, through both continued support for general health and social development and specific support to national and international

prevention and control programmes, including emergency control;

REQUESTS the Director-General:

- (1) to develop further and support implementation of the global strategy for prevention and control of dengue fever and dengue haemorrhagic fever through integrated environmental management;
- (2) to continue to seek resources for advocacy and research on improved and new tools and methods for dengue fever prevention and control and their application;
- (3) to study the need for and feasibility of incorporating the surveillance and research of other arthropod-borne viral infections, such as Japanese encephalitis, West Nile, and other emerging diseases, in the surveillance system for dengue haemorrhagic fever;
- (4) to mobilize financial resources to be spent on vector control and research into vaccines.

*Ninth plenary meeting,  
18 May 2002*

## Instructions for Contributors

The *Dengue Bulletin* welcomes all original research papers which have a direct or indirect bearing on dengue fever/dengue haemorrhagic fever prevention and control, including case management. Papers should not contain any political statement or reference. In addition to full papers, the *Bulletin* publishes short notes, review articles and book reviews.

Manuscripts should be typewritten in English in triple space on one side of white A4 size paper, with a margin of at least 4 cm. on either side of the text and should not exceed 15 pages. The title should be as short as *possible*. The name of the author(s) should appear after the title, followed by his or her official position, name of institution and complete address.

References to published works should be listed on a separate page at the end of the paper. References to periodicals should include the following elements: name and initials of author(s); title of paper or book in its original language; complete name of the journal, publishing house, or institution concerned; volume and issue number, relevant pages and date of publication, and place of publication (city and country). References should appear in the text in the same numerical order (Arabic numbers in parenthesis) as at the end of the article. For example:

- Nimmannitaya S. Clinical spectrum and management of dengue haemorrhagic fever. The Proceedings of the International Conference on Dengue Haemorrhagic Fever, Kuala Lumpur, September 1-3, 1983:16-26.
- World Health Organization. Viral haemorrhagic fevers. Report of a WHO Expert Committee, Technical Report Series 721, 1985.
- Jamaluddin M, Jalees S, Sharma RS and Verghese T. Dengue/DHF outbreak in Shahjahanpur, Uttar Pradesh, India. Dengue Newsletter, 1993, 18:2.

Figures and tables (Arabic numerals), with appropriate captions and titles, should be included on separate pages, numbered consecutively, and attached at the end of the text with instructions as to where they belong.

Articles should include an abstract of not more than 300 words conveying the content of the paper and its main conclusions; an introduction explaining clearly why the work described was carried out and what it is expected to contribute to scientific and technical knowledge; and conclusions and recommendations, if pertinent.



Articles submitted for publication should be accompanied by a statement that they have *not* already been published, and, if accepted for publication in the Bulletin, will not be submitted for publication elsewhere without the agreement of WHO, and that the right of republication in any form is reserved by the WHO Regional Offices for South-East Asia (SEARO) and the Western Pacific (WPRO).

One hard copy, original and clear figures/tables and a computer diskette indicating the name of the software, of the manuscript should be submitted to:

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