Dengue fever/dengue haemorrhagic fever (DF/DHF) continues to be a major infectious disease of public health importance in countries of the Western Pacific and South-East Asia regions. The two regions are experiencing a geographical spread, both in terms of the distribution of the viruses and the mosquito vector, with an increase in the frequency of epidemics. In 1999, as in 1998, several countries in the South-East Asia Region continued to report outbreaks of DHF. Although these countries have launched several community-based vector control programmes, they have achieved only varying degrees of success.

The WHO South-East Asia Regional Office has undertaken an external review of the national dengue control programme in Thailand to review the current situation, policies and strategies, the management and administrative aspects and policies and strategies, including resource mobilization and networking of partners to improve the output of the national programme.

The deadline for the receipt of contributions for the next issue of Dengue Bulletin (Volume 24) is 31 December 2000. Contributors are requested to follow the instructions carefully while preparing the manuscripts. Contributions must be accompanied by computer diskettes using MS Word for Windows and should be sent to the Editor, Dengue Bulletin, WHO/SEARO, Mahatma Gandhi Road, I.P. Estate, New Delhi-110 002, India; E-mail: dengue@whosea.org.

Readers desirous of obtaining copies of the Dengue Bulletin may contact the respective WHO Regional Offices in New Delhi or Manila or the WHO Country Representative in their country or residence.

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Can Doctors Make an Accurate Diagnosis of Dengue Infections at an Early Stage?

By
S. Kalayanarooj*, S. Nimmannitya*, S. Suntayakorn*, D.W. Vaughn†, A. Nisalak*, S. Green‡, V. Chansiriwongs*, A. Rothman†, F.A. Ennis‡.

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Abstract
As part of a multi-centre, prospective study of dengue pathophysiology between 1994-1997, clinical findings and simple laboratory tests were evaluated to find early indicators for the diagnosis of dengue infections so that control actions could be taken as early as possible to prevent the spread of dengue in the community. Six hundred and forty-nine febrile children with a flushed face and without signs of localized infection were followed as in-patients. Three hundred and eighteen children were confirmed to have dengue: 176 dengue fever (DF), and 142 dengue haemorrhagic fever (DHF); another 331 children had other, self-limiting febrile illness (OFI).

Tourniquet test (TT) positive and leukopenia (WBC ≤ 5,000 cells/cu.mm.) were the two screening tests that helped in the early clinical diagnosis of dengue infections.

Studies revealed that TT positive or leukopenia were the two tests that had a high sensitivity of about 90% for the diagnosis of dengue patients, but their specificity and positive predictive value (PPV) were only 50-60% and 60-70% respectively. If the two tests are combined, the sensitivity gets reduced to 74% while the specificity and PPV are increased to 85% and 83% respectively. For early, effective and rapid control of dengue outbreak, TT or leukopenia is a good indicator for initiating immediate control measures. TT positive with leukopenia is also a good indicator for immediate control measures, 83% of this immediate control measures will be necessary but about 26% of dengue cases that have no TT positive with leukopenia will be missed.

Key words: Dengue haemorrhagic fever, Tourniquet test, Leukopenia, Thailand
Introduction

Dengue infections have been one of the major diseases affecting children in Thailand for more than 40 years. First dengue epidemic was recorded with 2,158 cases in 1958 and reached a peak in 1987 when there were 174,285 cases reported. The last two epidemics occurred in two consecutive years, 1997 and 1998, when 101,689 cases and 127,189 cases, respectively, were reported. Although the case-fatality rate has been reduced from 14% (1958) to 0.34% (1998), the number of deaths was higher, from 300 deaths in 1958 to 464 deaths in 1998. Adults were affected more than expected, and their share of deaths was to about 20% in 1998 (1).

During the last two epidemics, one of the major reasons for not taking control measures was the delay in case reporting (2). This delay in reporting was due to clinicians being reluctant to report dengue haemorrhagic fever (DHF) cases without serological confirmation. The disease control authorities were doubtful about the clinical diagnosis as most of the criteria used was non-specific (3). They preferred to wait for confirmed cases before taking control actions.

This study is a part of the collaborative dengue pathophysiology studies and was planned to find simple clinical and/or laboratory indicators for the early diagnosis of dengue infections that would help speed up the reporting system so that control actions would start early and be effective to arrest the spread of the outbreak.

Materials and methods

Twelve febrile patients were enrolled each week between 1994 and 1997 from the outpatient department of two hospitals, Children’s Hospital in Bangkok and Kamphangpet Provincial Hospital. The patients met the following criteria: age 6 months to 15 years, had temperature ≥ 38.5°C Celsius for < 72 hours, had facial flushing and no obvious source of infection. Parents or guardians of all patients had to sign an informed consent before participating in this project. Patients who had signs of shock or had underlying diseases were excluded from the study.

All the patients were admitted to hospital for close observation. Study physicians did the history-taking and physical examination, including tourniquet test (TT), everyday. Daily phlebotomy was done every morning for CBC, dengue serological (ELISA and Haemagglutination Inhibition test), virological (mosquito inoculation technique) and immunological study for five days or until one day after defervescence (whichever came first). Right lateral decubitus chest films to detect pleural effusion were done one day after defervescence. Blood studies were repeated on study day 9 when the patients came for a follow up. Liver function test and coagulogram were studied on the first study day, on the day of defervescence or one day after and at the time of follow-up (2,3,4,5).

Patients were classified as DF and DHF according to the WHO criteria (6,7). DHF severity was also classified according to the WHO criteria. Patients with bacterial or other definite infections other than dengue were excluded from the study. Patients with self-limited febrile illness without definite sources of infection were classified as other febrile illness (OFI).
Comparisons between the clinical and simple laboratory indicators for the two groups of patients, DHF/DF and OFI, were done. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each indicator(s) for the diagnosis of dengue infection were calculated using serological and/or virological tests as the standard diagnosis of dengue infections \(^5\).

### Results

#### Patients’ profile

There were 649 patients who were eligible for this study. Among the 318 dengue patients, 176 were of DF and 142 of DHF (42 DHF grade I, 78 DHF grade II, and 22 DHF grade III). There were 331 patients in the OFI group. The male/female ratios for dengue and OFI patients were 1:1.12 and 1:1.45, respectively. The mean ages for DF, DHF grade I, DHF grade II, DHF grade III and OFI patients were 8.01 (± 2.93), 8.25 (± 3.58), 8.9 (± 2.86), 7.48 (± 2.4) and 6.59 (± 3.15) years, respectively.

#### Serology

A total of 313 patients (98.43%) were confirmed serologically: 21.09% were primary while 78.91% were secondary dengue infections. Primary and secondary dengue infections were found in 30.64% and 69.36% of DF patients while 9.29% and 90.71% of the primary and secondary dengue infections were found in DHF patients (Table 1).

#### Virology

A total of 302 patients (94.96%) had dengue viral isolation: 39.74% were type 1, 23.18% type 2, 28.15% type 3, and 8.61% were type 4, while 0.33% could not be identified (Table 2).

#### Fever

The mean duration of fever for DF, DHF grade I, DHF grade II, DHF grade III and OFI patients was 4.08 days (± 1.19), 4.51 days (± 0.90), 4.38 days (± 0.99), 5.27 days (± 1.72) and 3.13 days (± 1.69), respectively. Among DHF patients 2.16% had fever for 2 days, 10.07% had fever for 3 days, 41.01% had fever for 4 days, 30.94% had fever for 5 days, 11.51% had fever for 6 days and 2.16% had fever for 7 days (Figure 1).

### Table 1. Serological results in dengue patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Primary (%)</th>
<th>Secondary (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>53 (30.64)</td>
<td>120 (69.36)</td>
<td>173 (55.27)</td>
</tr>
<tr>
<td>DHF</td>
<td>13 (9.29)</td>
<td>127 (90.71)</td>
<td>140 (44.73)</td>
</tr>
<tr>
<td>Total</td>
<td>66 (21.09)</td>
<td>247 (78.91)</td>
<td>313 (100)</td>
</tr>
</tbody>
</table>

### Table 2. Virus isolation

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Den 1 (%)</th>
<th>Den 2 (%)</th>
<th>Den 3 (%)</th>
<th>Den 4 (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>75 (39.74)</td>
<td>29 (23.18)</td>
<td>47 (28.15)</td>
<td>15 (8.61)</td>
<td>166</td>
</tr>
<tr>
<td>DHF</td>
<td>15 (45)</td>
<td>41 (41)</td>
<td>38 (38)</td>
<td>26 (26)</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>70</td>
<td>85</td>
<td>26</td>
<td>301</td>
</tr>
</tbody>
</table>
Can Doctors Make an Accurate Diagnosis of Dengue Infections at an Early Stage?

**Figure 1.** Duration of Fever

<table>
<thead>
<tr>
<th>Days</th>
<th>OFI</th>
<th>DF</th>
<th>DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2.16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>11.51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>30.94</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>41.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>11.91</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>1.44</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 2.** Tourniquet test

<table>
<thead>
<tr>
<th>Days</th>
<th>OFI</th>
<th>Dengue</th>
<th>DF</th>
<th>DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4</td>
<td>27.78</td>
<td>45.59</td>
<td>35.39</td>
<td>46.97</td>
</tr>
<tr>
<td>-3</td>
<td>40.71</td>
<td>55.56</td>
<td>35.39</td>
<td>46.97</td>
</tr>
<tr>
<td>-2</td>
<td>67.82</td>
<td>55.56</td>
<td>35.39</td>
<td>46.97</td>
</tr>
<tr>
<td>-1</td>
<td>77.82</td>
<td>43.2</td>
<td>55.33</td>
<td>46.97</td>
</tr>
<tr>
<td>0</td>
<td>89.92</td>
<td>100</td>
<td>55.33</td>
<td>46.97</td>
</tr>
<tr>
<td>1</td>
<td>90.88</td>
<td>100</td>
<td>55.33</td>
<td>46.97</td>
</tr>
</tbody>
</table>
**Tourniquet test**

The percentage of TT positive (≥ 10 petechiae/square inch) in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients was 87.50%, 90.48%, 94.87%, 90.91% and 51.96%, respectively.

In dengue patients TT was positive in 45.59%, 55.56%, 67.27% and 77.82% of cases on day 4, 3, 2, 1 before defervescence and 89.92% on the day of defervescence. DHF patients had a much higher percentage of TT positive as compared to DF patients (Figure 2).

The sensitivity, specificity, PPV and NPV for TT for the diagnosis of dengue infections were 89.94%, 48.04%, 62.45% and 83.25%, respectively (Table 3).

<table>
<thead>
<tr>
<th>TT positive</th>
<th>Leuko-</th>
<th>TT positive + leukopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>89.94</td>
<td>91.19</td>
</tr>
<tr>
<td>Specificity</td>
<td>48.04</td>
<td>59.82</td>
</tr>
<tr>
<td>PPV</td>
<td>62.45</td>
<td>68.56</td>
</tr>
<tr>
<td>NPV</td>
<td>83.25</td>
<td>87.61</td>
</tr>
</tbody>
</table>

**WBC**

The mean WBC in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients were 4,929 (± 2,705); 5,147 (± 2,667); 4,808 (± 2,420); 5,576 (± 2,969) and 8,754 (± 4,860) cells/cu.mm, respectively.

---

**Table 3. Comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) between dengue and OFI patients in the diagnosis of dengue infections**

**Figure 3. Mean WBC count**
The mean WBC in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients one day before defervescence were 3,834 (± 2,216); 3,870 (± 1,553); 3,257 (± 1,583); 4,595 (± 2,415) and 9,743 (± 6,391) cells/cu.mm, respectively (Figure 3).

The percentage of leukopenia (≤ 5,000 cells/cu.mm) found in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients was 89.77%, 95.24%, 92.31%, 90.91% and 40.18%, respectively.

The sensitivity, specificity, PPV and NPV for leukopenia (≤ 5,000 cells/cu.mm) for the diagnosis of dengue infections were 91.19, 59.82, 68.56 and 87.61%, respectively (Table 3).

**Tourniquet test and leukopenia (≤ 5,000 cells/cu.mm)**

The percentage of TT positive and leukopenia found in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients was 72.16%, 73.81%, 80.77%, 68.18% and 14.50%, respectively.

The sensitivity, specificity, PPV and NPV for TT positive and leukopenia for the diagnosis of dengue infections were 74.21%, 85.50%, 83.10% and 77.53%, respectively (Table 3).

**Aspartate aminotransferase (AST)**

The mean values of AST in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients were 61.65 (± 56.36), 68.45 (± 52.20), 99.04 (± 112.61), 162.43 (± 222.14) and 38.05 (± 18.42) units(U), respectively.

The percentage of AST > 40 U in DF, DHF and OFI patients was 90.91%, 98.59% and 57.1%, respectively (Figure 4).

The percentage of AST > 60 U in DF, DHF and OFI patients was 63.07, 92.96 and 15.1%, respectively.

The sensitivity, specificity, PPV and NPV for AST (≥ 60U) for the diagnosis of dengue infections were 76.42%, 84.89%, 82.89% and 79.93%, respectively.

**Alanine aminotransferase (ALT)**:

The mean values of ALT in DF, DHF grade I, DHF grade II, DHF grade III and OFI patients were 33.71 (± 28.77), 32.43 (± 21.31), 53.09 (± 59.98), 69.45 (± 80.27) and 21.72 (± 14.27) units(U), respectively.

The percentage of ALT > 40 U in DF, DHF and OFI patients was 53.98%, 77.47% and 12.69%, respectively (Figure 5).

The percentage of ALT > 60 U in DF, DHF and OFI patients was 28.98%, 47.89% and 5.10%, respectively.

The sensitivity, specificity, PPV and NPV for ALT (≥ 60U) for the diagnosis of dengue infections were 37.42%, 94.86%, 87.50% and 61.21%, respectively.

**Discussion**

Males and females are affected equally by dengue viruses. The peak age of contracting dengue infection was between 5 and 9 years. As reported earlier, 90.71% of DHF patients in this study had secondary dengue infection while 69.36% of DF patients had secondary dengue infection.
Can Doctors Make an Accurate Diagnosis of Dengue Infections at an Early Stage?

Figure 4. Percentage of patients with elevation of AST

Figure 5. Percentage of patients with elevation of ALT
All the four dengue serotypes were found. DEN-1 was more predominant while DEN-4 was less common.

OFI patients tended to have a shorter duration of fever (3 days) than dengue patients (4-5 days). The shortest duration of fever for DHF patients was 2 days (2.16%). Most of the DHF patients (72%) had fever for 4-5 days. This is very important and needs to be emphasized to the clinician that the earliest day of shock in DHF patient could be on day 3 of the illness, i.e. one day after defervescence, and the critical day for most DHF patients would be the fifth or sixth day of their dengue illness.

TT positive alone has the sensitivity of 89.94% for the diagnosis of dengue infections as compared to the previous finding of 97.1%. PPV of TT positive is 62.45%. Specificity is 48.04% as compared to the previous report of 97.2% because 51.96% of other viral infections in this study had positive TT while only a few cases of viral infection were included in the previous study. Experienced clinicians however can differentiate dengue clinically from other viral infections on the basis of TT positive as most of dengue patients have bigger petechiae size as compared to the very fine petechiae in other viral infections. About half of the dengue patients had positive TT on the first day of illness. The percentage of TT positive in dengue patients increased everyday and 78% had positive TT one day prior to defervescence, which could help clinicians to make presumptive clinical diagnosis before they entered into critical periods. (About 90% of DHF patients had TT positive before they had defervescence.)

The mean WBC in dengue patients was lower than in the OFI group. A mean WBC of ≤ 5,000 cells/cu.mm was found in all DHF patients one day prior to defervescence as reported previously, so this is a good indicator to warn clinicians that the patient is near to the critical stage of the disease. Also, leukopenia of ≤ 5,000 cells/cu.mm had the sensitivity for the diagnosis of dengue infection = 91.19%, specificity = 59.82%, and PPV = 68.56%. Although leukopenia had a little bit better sensitivity, specificity and PPV than TT, but it occurred much later in the course of the illness as compared to the occurrence of positive TT.

When we combined the findings of TT positive and leukopenia, the sensitivity for the diagnosis of dengue infection was reduced to 74.21%, but the specificity and PPV increased to 85.50% and 83.19%, respectively.

Since 63.07% and 92.96% of DF and DHF patients had AST > 60 U while only 15.1% of OFI patients had it, AST is also a good indicator for differentiating dengue from other viral illnesses. AST elevation occurred as early as in the first few days of the illness. AST > 60 U had the sensitivity, specificity and PPV of 76.42%, 84.89% and 82.89%. AST cannot be done in all small hospitals, so it may not be as useful as TT or leukopenia for early diagnosis of dengue infection. When DHF patients present with encephalo-pathy, AST/ALT should be done. If the values exceed 200 U, they suggest that the patient may have hepatic dysfunction/ hepatic encephalopathy.
In this study, very few dengue patients had petechiae so it did not help in early clinical diagnosis. Platelet counts ≤ 100,000 cells/cu.mm occurred late in the course of dengue illness, which did not help in early diagnosis.

**Conclusion**

From this study it is evident that TT positive is the earliest test that occurs in dengue illness to make clinicians think of dengue infection. If they report dengue infection as soon as they find TT positive, and control activities are initiated within 24 hours, it is most likely that the spread of dengue outbreak can be checked and controlled without much damage. Clinicians should also report dengue infection when they see leukopenia in febrile patients (leukopenia usually occurs later than TT positive). If control actions are taken as soon as TT positive or leukopenia are observed, DHF outbreaks can be substantially controlled up to 90% (sensitivity), but the control activities may be unnecessary in the presence of 31% for leukopenia and 38% for TT (100-PPV).

When outbreak control activities are undertaken with the finding of TT positive and leukopenia together, which occur later than TT positive or leukopenia alone, the control actions will provide only 74.21% (sensitivity) of coverage for dengue infection, but the actions are unnecessary at 14% level (100-PPV).

To control dengue outbreaks more effectively, we suggest that clinicians should report dengue cases as soon as they find TT positive because it is most rapid and has the highest sensitivity. Nevertheless, control strategies for dengue outbreak should be thoroughly examined by the authorities in order to get the maximum benefit for the manpower and the budget available in that time frame.

**References**

Standardized Clinical Management: Evidence of Reduction of Dengue Haemorrhagic Fever Case-Fatality Rate in Thailand

By
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Abstract
The first outbreak of dengue haemorrhagic fever (DHF) in Thailand occurred in 1958. There were 2,158 cases reported and 300 deaths, with a case-fatality rate (CFR) of 13.9%. Guidelines for the diagnosis and management of DHF were developed at the Children’s Hospital, Bangkok, by Dr Suchitra Nimmannitya a few years after the outbreak. These guidelines were used widely in Thailand and have resulted in a marked reduction in the CFR from 14% to less than 1%. WHO adopted these criteria for the diagnosis and management of DHF in 1975 and has distributed it for worldwide use. The prognosis of DHF patients depends on early diagnosis and early detection of shock with proper management. This paper emphasizes on tourniquet test which is an important screening test for the diagnosis of dengue infection. CBC is an important laboratory test to be followed frequently in these patients. Leukopenia < 5,000 cells/cu mm and lymphocytosis indicate that the critical period is approaching within 24 hours and that warning signs and symptoms of shock should be told to the care-takers and they should be persuaded to bring the patient back to the hospital as soon as possible. Proper IV fluid management when the patients are in a critical period of 24-48 hours (when platelets are < 100,000 cells/cu mm and 10-20% rising hematocrit) are explained in detail. Most DHF patients (60-70%) require only crystalloid solution (isotonic salt solution). Only 15-20% have massive plasma leakage and need colloidal solution for which dextran-40 with its hyperoncogenicity (about 3 times that of plasma) is recommended. Ten to 15% of DHF patients need blood transfusion. Platelet transfusion is recommended only for those with severe bleeding. With good medical and nursing care and appropriate management, all patients should recover rapidly and completely.

Key words: Dengue haemorrhagic fever, Tourniquet test, Leukopenia, Fluid Management, Colloidal solution
**Introduction**

The first case of dengue haemorrhagic fever (DHF) in Thailand in 1950 was diagnosed as “influenza with bleeding”, and the first DHF epidemic occurred in 1958 when it was limited only to Bangkok. There were 2,158 cases reported with 300 deaths, and with a morbidity rate (MR) of 8.87/100,000 population and case-fatality rate (CFR) of 13.9%\(^1\).

Guidelines for the diagnosis and management of DHF were developed at the Children’s Hospital by Dr Suchitra Nimmannitya.\(^2\) The use of these guidelines brought down the CFR from 13.9% to 5% in the first 8 years. After this period, the CFR was reduced gradually from 5% to 1% in about 10 years due to the spread of DHF to most big cities in the country. Since 1971, the number of reported cases has been continuously on the increase. However, CFR has stayed <1% since 1979 and was below 0.5% in 1989\(^1\).

**Critical areas in efficient case management**

The clinical and laboratory criteria for the diagnosis of DHF developed at the Children’s Hospital, Bangkok, during 1975\(^5\) and which has been adopted by WHO, are based on the presence of major manifestations, in order of their appearance:

1. High continuous fever for 2-7 days.
2. Haemorrhagic manifestations, including at least a positive tourniquet test.
3. Enlargement of liver.
4. Circulatory disturbances (as shock in severe cases).
5. Thrombocytopenia ≤ 100,000 cells/ cu.mm.
6. Haemoconcentration: hematocrit (Hct) increased by 20% or other evidence of plasma leakage i.e. pleural effusion and/or ascites.

These criteria meet 95% confidence level for making the diagnosis of DHF, but the diagnosis can be made only when a patient has completed his clinical course of illness.

DHF is classified into four grades according to the clinical hallmarks of bleeding and shock. Most patients of DHF grade I and II (non-shock) can recover spontaneously or shortly after a brief period of fluid therapy. In contrast, DHF grade III, and especially grade IV, patients need special attention and care from physicians and nurses with appropriate fluid resuscitation and judicious volume replacement. Correction of any metabolic and/or electrolytes abnormalities are critical in these patients. Concealed internal bleeding is likely in patients with prolonged shock.

Early detection of shock and proper management are the most important factors that determine the prognosis of DHF patients.

The clinical course of DHF is divided into three phases:

1. Febrile phase (2-7 days)
2. Critical or leakage phase (24-48 hours)
3. Convalescence phase (2-7 days)

The management of DHF cases\(^4,5,6\) is divided according to these three phases.
1. **Febrile phase**

(a) Screen all suspected dengue patients.

- **Tourniquet test** is an important tool for early screening of dengue patients from other viral/bacterial illnesses. It should be done in all children with high fever, flushed face, and without any focal signs and symptoms of infection. A positive tourniquet test is ≥ 10 dots/square inch. The tourniquet test is positive in 50% of the patients on the first day, in 60-70% on the second day and >90% on the third day onwards.

(b) Supportive and symptomatic treatment.

- Give paracetamol 10 mg/kg/dose prn T ≥ 39°C q 4-6 hr., aspirin and ibuprofen are contraindicated.
- Apply tepid sponges if a patient still has high fever after a dose of paracetamol.
- For nutritional support, advise soft diet, fruit juice, milk or ORS.

(c) If a suspected patient has signs of dehydration and had severe vomiting, give 5% D/N/2 to correct dehydration and discontinue IV fluid as soon as possible, preferably within 24 hours. If IV fluid cannot be discontinued, give only a minimal amount - about half of the maintenance amount.

(d) Follow all suspected dengue patients closely everyday from day 3 of their illness.

During the febrile phase, it is difficult to differentiate between DF and DHF patients because they have almost the same clinical symptoms, except that maculopapular rash and myalgia/arthritis are less frequent in DHF. So one has to follow carefully all suspected dengue infected patients until they are afebrile for 24 hours without the use of antipyretic. At the end of the febrile phase, DF patients will recover spontaneously while in DHF patients, the critical stage is reached. In mild DHF cases, the change in vital signs is minimal and transient. Patients will recover spontaneously or shortly after intravenous fluid administration. In more severe cases, the disease progresses rapidly into the stage of shock.

To follow these patients closely:

- Ask the history of bleeding (admit and give blood transfusion if there is a significant amount of blood loss, ~10% of total blood volume).
- Do physical examination:
  - Vital signs - signs of shock: rapid and weak pulse, narrowing of pulse pressure, hypotension - if present, give IV fluid immediately and admit.
  - Palpate the liver - liver enlargement and tenderness indicate nearness to or entering the critical phase; observe closely or admit.

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* Tourniquet test is performed by inflating a blood pressure cuff on the upper arm to a point midway between the systolic and diastolic pressure for five minutes. Wait for one minute after the release of pressure before reading the test.
Repeat tourniquet test if it was negative.

- Do CBC and follow CBC everyday:
  - Leukopenia $\leq 5,000$ cells/cu.mm. and lymphocytosis, increase in atypical lymphocyte indicates that within the next 24 hours, the patient will have no fever (7) and will enter critical phase if they are DHF cases.
  - Thrombocytopenia $\leq 100,000$ cells/cu.mm. indicates that the patient is entering a critical phase and needs close observation in the hospital.
  - Rising Hct 10-20% indicates that the patient is in the critical period and needs IV fluid therapy if he cannot have good oral intake. Admit this patient and give isotonic salt solution.

(e) Advise caretakers about warning signs of shock so that parents should bring their children to the hospital as soon as possible.

- Clinical deterioration when defervescence occurs.
- Bleeding.
- Acute, severe abdominal pain/vomiting.
- Very drowsy, patient looks weak and sleeps all the time.
- Refuses to eat or drink (some may complain of being very thirsty).

- Restless, irritable.
- Change in behaviour.
- Cold, clammy or mottling skin.
- Not passed urine for 4-6 hours.

(f) Indications for admission

- Very weak and cannot have adequate oral intake.
- Bleeding.
- Platelet $\leq 100,000$ cells/cu.mm. and/or rising Hct 10-20%.
- Clinical deterioration when defervescence occurs.
- Acute, severe abdominal pain/vomiting.
- Shock/impending shock.
- Rapid pulse and no fever.
- Capillary refill $>2$ seconds.
- Cold, clammy skin, mottling, restless.
- Pulse pressure $\leq 20$ mmHg, e.g. 100/80, 90/70.
- Hypotension.
- No urine for 4-6 hours.
- Change of consciousness, stuporous or aggressive behaviour which may indicate more severe disease, encephalopathy.
- Parental anxiety, live far away from the hospital.

2. Critical/Leakage phase

Most of the admitted cases are more severe patients who cannot have adequate oral
intake, whether anorexia and/or vomiting during the critical/leakage phase.

(a) General management of patients

- Put all dengue patients together in the dengue ward or dengue corner for close observation. This ward should have mosquito net to prevent nosocomial dengue transmission.

- Vital signs should be measured q 1-2 hours; if unstable vital signs are present, it should be done more frequently, i.e. q 10-15 minutes.

- Hct should be done q 4-6 hours; if unstable vital signs and suspected internal bleeding, more frequently, i.e. q ?-1 hour. This is very critical, especially in cases with concealed bleeding.

- Record intake/output.

- Should have flow chart at bedside for recording clinical signs and symptoms, vital signs, Hct, intake/output, which is very important for adjusting the rate and type of fluid therapy.

- Give oxygen via face mask/nasal canular in cases with shock.

- Stop bleeding by proper methods, e.g. nasal packing in cases with epistaxis.

- Avoid unnecessary invasive procedures, e.g. do not insert naso-gastric tube in cases with upper GI bleeding.

- Closely observe the patients by both physicians and nurses.

(b) High risk patients

The following types of patients are at risk, so nurses should notify attending staff as soon as possible. These patients need special laboratory investigations for they may have complications, e.g. internal bleeding, hypoglycemia, electrolyte imbalance (hyponatremia, hypocalcemia), metabolic acidosis, liver failure and renal failure. (The lab. investigations include, Hct, blood sugar, electrolyte + Ca, capillary or venous blood gas, coagulogram, liver function test, BUN and creatinine). These patients are:

- Young infants < 1-year old.
- DHF grade IV or prolonged shock.
- Overweight patients.
- Patients with massive bleeding.
- Patients with changes of consciousness (encephalopathy).
- Patients with underlying diseases, e.g. thalassemia, G-6-PD deficiency, congenital heart disease, etc.
- Referred patients.

(c) Fluid management

Indication for IV fluid

- Thrombocytopenia <100,000 cells/ cu.mm., rising Hct 10-20%, platelet ≤ 100,000 cell/cu.mm. and patient cannot have adequate oral intake.

- Shock or impending shock.
Type of IV fluid

- Isotonic salt solution, e.g. Acetate Ringer (AR), Lactate Ringer (LR) or normal saline solution (NSS) with or without 5% dextrose (preferably with 5% dextrose).
- In young infants, during shock use isotonic solution; if not in shock, use 5% D/N/2.

Amount of IV fluid:

- During the critical period of plasma leakage (24-48 hours), DHF patients should receive the total amount of IV fluid for maintenance + 5% deficit (M + 5% D). Based on the observation that the average amount of IV fluid given through the period of leakage in DHF grade III patients is equal to that.
- In patients whose body weight is more than 40 kg, the total amount of IV fluid should be equal to 2 times the maintenance (2M) (because 2M is less than M + 5% D).
- In overweight patients, calculate IV fluid according to the ideal body weight (BW) [(BW X 2) + 8].
- In adults, calculate IV fluid based on average BW of 50 kg.

Rate of IV fluid

- In non-shock cases, start with:
  - 5 ml/kg/hr. (BW between 15-40 kg).
  - 3-4 ml/kg/hr. (BW > 40kg.).
  - 6-7 ml/kg/hr. (BW < 15 kg).
- In DHF grade III, start with 10 ml/kg/hr.
- In DHF grade IV, start with 10 ml/kg IV push or drip free flow for 10-15 minutes until blood pressure (BP) and pulse (P) can be measured, then reduce to 10 ml/kg/hr.

Adjusting the rate of IV fluid

It is very important to adjust the rate of IV fluid frequently to avoid fluid overload. In DHF patients, IV fluid should be given at the minimal amount to keep intravascular circulation because if more IV fluid is given, it will leak out into both the pleural and abdominal cavities and cause respiratory distress later. The rate of IV fluid should be adjusted according to:

- Clinical conditions: general appearance, capillary refill, appetite.
- Vital signs: BP, P, temperature (T) and respiratory rate (RR).
- Hct.
- Urine output.

The first 6-12 hours after shock, BP and P are the two important parameters to determine the rate of IV fluid, but later, consider all parameters together before adjusting the rate.

(d) Monitoring of shock

- After initial fluid resuscitation, evaluate the patient at 1-2 hours. If the rate of IV fluid cannot be reduced to <10 ml/kg/hr. because of unstable
vital signs (still narrowing of pulse pressure and rapid and weak pulse), Repeat the Hct:

- If there is an increase, change IV fluid to colloidal solution (Dextran-40 is preferred) at a rate of 10 ml/kg/hr.
- If there is a decrease, change IV fluid to colloidal solution (Dextran-40 is preferred) at a rate of 10 ml/kg/hr. and cross match for fresh whole blood (FWB) and re-evaluate the patient again after one hour whether he needs blood transfusion or not.
- In grade IV patients,
  - If the initial Hct is very low, i.e. 40%-45%, think of possible internal haemorrhage and follow Hct more frequently and give blood transfusion as soon as indicated.
  - Correct the possible metabolic and electrolyte disturbance, e.g. hypoglycemia, hyponatremia, hypocalcemia, acidosis.
  - After 6 hours, if Hct is decreasing and in spite of a large amount of volume replacement, still cannot reduce the rate of IV fluid to < 10/ml/kg/hr., consider blood transfusion as soon as possible.

The recommended colloidal solution

- Dextran-40 (10% dextran-40 in NSS which is a plasma expander) is recommended because of its hyperoncogenicity (osmolarity ~ 3 times that of plasma), so it can hold the volume better. Other colloidal solutions, including plasma itself, are the plasma substitute and have osmolarity ~ 1-1.4 times that of plasma.
- The rate of dextran-40 should be 10 ml/kg/hr so that it can maintain maximum osmolarity when administered to the patients.
- The maximum dosage of dextran-40 is 30 ml/kg/day. Do not exceed this amount for it may cause acute renal failure.

Duration of IV fluid

- The duration of IV fluid administration should not exceed 24-48 hours.
- Indication for blood transfusion
  - Significant amount of blood loss, i.e. > 10% of total blood volume (TBV). TBV = 80 ml/kg. Give FWB replacement equal to the amount observed.
  - Patients with haemolysis due to their underlying diseases, e.g. G-6-PD deficiency, thalassemia.
  - Patients with concealed bleeding. Hct dropped and unstable vital signs in spite of large amount of volume replacement, give FWB 10 ml/kg/dose or pack red cell (PRC) 5 ml/kg/dose at a time.

Indication for platelet transfusion

Platelet concentrate is indicated only in cases with massive bleeding. Give 0.2 unit/kg/dose.
3. **Convalescence phase**

In general, most DHF patients will recover rapidly without complication within 24-48 hours after shock. Indicators for recovery include:

- Improved general condition.
- Gain appetite.
- Stable vital signs: wide pulse pressure, slow and strong pulse.
- Hct stable and decrease to baseline value, 35%-40%.
- Diuresis.
- Some (~ 30%) developed confluent petechial rash with characteristic, scattered small, whitish round areas on their lower extremities, may be itchy.
- Sinus bradycardia.

IV fluid should be discontinued immediately if they enter convalescence phase. If the patient does not gain appetite and has distended abdomen with decreased or no bowel sound, check for hypokalemia which is commonly found in this phase (due to diuresis). Fruit or fruit juice or KCl solution orally are recommended to correct this electrolyte abnormality.

4. **Indications for discharge**

- At least 24 hours after defervescence without using antipyretic.
- Good appetite.
- Visible good general conditions.
- Diuresis.
- Stable Hct at baseline value 35%-40%.
- At least 2 days after shock.
- No dyspnea or tachypnea.
- Platelet ≥ 50,000 cell/cu.mm.
- No complications.

**References**


Use of Predicted Amino Acid Sequence of Envelope-Nonstructural Protein 1 Region to Study Molecular Evolution of Epidemic-Causing Dengue-2 Strains

By
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Abstract
In the present study a quantitative comparison of amino acid sequence from envelope-nonstructural protein 1 gene junction region of 10 dengue virus type 2 (DEN-2) isolates from Delhi with 12 DEN-2 isolates from diverse geographical areas and hosts provided sufficient information for estimating genetic relationships. The data indicated that the 1996 epidemic of DHF in Delhi was caused by genotype IV strains of DEN-2. This genotype displaced genotype V strains of DEN-2, which was the circulating genotype in 1967. The period during which this displacement had occurred is not clear from the present study. Nonetheless, similar experience in four countries in Latin America and in Sri Lanka suggests that the introduction of new genotypes of DEN-2 displacing the circulating genotype may be associated with the appearance of DHF/DSS. More work is required to elucidate this hypothesis. However, no segregation of virus strains was observed in terms of the severity of the clinical presentation of dengue virus infections (DF/DHF/DSS). The data presented in this study suggest the utility of phylogenetic analysis of the amino acid sequence of E-NS1 junction region for molecular epidemiology of dengue viruses.

Key words: Dengue virus, amino acid sequence genotype, molecular epidemiology, Delhi

Introduction
A widespread outbreak of dengue fever/dengue haemorrhagic fever (DF/DHF) swept across four states of north-western India: Punjab, Haryana, Delhi and Rajasthan during August-November 1996\(^1\,^{2}\). In Delhi alone more than 10,000 patients were
admitted in various hospitals and 423 died. The causative agent was identified as dengue virus type 2 (DEN-2). This was the first major epidemic of DHF in Delhi due to DEN-2, although frequent outbreaks of DF had been reported since 1967.

DHF has been postulated to result from immune enhancement after a second heterologous dengue (DEN) infection. However, reports of primary infections resulting in DHF suggest that differences in the virulence of DEN strains may also be involved. Further, molecular characterization of DEN isolates has suggested the existence of intra-serotypic genetic variants (genotypes). Movement of these genotypes between different geographical areas is an important element in the epidemiology of the disease. It has been postulated that the introduction of new genotypes of existing serotype into areas where dengue activity is troublesome could be associated with the severe form of the disease.

In this report, we analysed the predicted amino acid sequence of the envelope-nonstructural protein 1 (E-NS1) region of nine DEN-2 isolates from the Delhi epidemic of 1996 and one isolate from the Delhi epidemic of 1967 and compared the data with other published sequences of DEN-2 isolated from other parts of the world.

**Materials and methods**

The nucleotide sequence of E-NS region (2050-2442) of all the ten viruses from Delhi have been published and deposited with the Genbank (Table 1). The predicted amino acid sequence was obtained by the DNASIS programme (Hitachi Software Engineering, Yokahama, Japan). The sequences were aligned manually. The phylogenetic analysis including estimation of distances by p-distance method, bootstrapping and generation of a neighbour-joining tree were performed with the Molecular Evolutionary Genetics Analysis package (MEGA). Amino acid sequences were compared with those of DEN-2 strains from different geographical areas that have been previously published and downloaded from Genbank (Table 2).

**Results**

The predicted amino acid (aa) sequence from aa position 372 to 502 of the E-NS1 region of all the viruses listed in Tables 1 and 2 are shown in Figure 1. The sequences were aligned to DEN-2 prototype strain, New Guinea C (D2-NG). An analysis of the sequence data shows a divergence of 0% to 3% (mean 1%) among the 1996 Delhi epidemic strains. Five isolates, 838, 841, 979, 980 and 1432, had identical amino acid sequences whereas isolates 1029, 1430, 1436 and 1451 showed a scattered substitution of amino acids. However, there appears to be no particular region of hypervariability. These isolates were taken from the patients who were infected in the beginning, the peak and toward the end of the epidemic and were suffering from DF/DHF (Table 1). No significant difference was observed at the amino acid sequence level to correlate with the severity of the disease in these patients.
Table 1. Dengue-2 virus isolates Delhi epidemics of 1996 and 1967

<table>
<thead>
<tr>
<th>Virus Isolate ID#</th>
<th>Date of receipt of sample</th>
<th>Age (Yrs) / Sex</th>
<th>Diagnosis</th>
<th>Genbank Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>838</td>
<td>16/09/96</td>
<td>7/F</td>
<td>DF</td>
<td>AF 047403</td>
</tr>
<tr>
<td>841</td>
<td>15/09/96</td>
<td>7/M</td>
<td>DHF</td>
<td>AF 047404</td>
</tr>
<tr>
<td>979</td>
<td>26/09/96</td>
<td>16/F</td>
<td>DF</td>
<td>AF 047405</td>
</tr>
<tr>
<td>980</td>
<td>26/09/96</td>
<td>9/M</td>
<td>DHF</td>
<td>AF 047406</td>
</tr>
<tr>
<td>1026</td>
<td>30/09/96</td>
<td>18/M</td>
<td>DF</td>
<td>AF 047407</td>
</tr>
<tr>
<td>1430</td>
<td>24/10/96</td>
<td>20/F</td>
<td>DHF</td>
<td>AF 047408</td>
</tr>
<tr>
<td>1432</td>
<td>24/10/96</td>
<td>20/F</td>
<td>DF</td>
<td>AF 047409</td>
</tr>
<tr>
<td>1436</td>
<td>31/10/96</td>
<td>58/M</td>
<td>DHF</td>
<td>AF 134980</td>
</tr>
<tr>
<td>1967</td>
<td>Not known</td>
<td></td>
<td>DF</td>
<td>AF 047410</td>
</tr>
</tbody>
</table>

When these strains were compared with other DEN-2 isolates, it was observed that the 1996 Delhi epidemic strains resembled strain 8730 from Seychelles, which was isolated from a patient with DHF in 1977 and differed from all other strains analysed in having serine substituting for asparagine at amino acid position 390 of the envelope region (Fig 1). Nonetheless, all the 1996 Delhi epidemic strains had asparagine in place of serine at position 502 of the NS1 gene region.

Table 2. Global Dengue-2 viruses used for amino acid sequence analysis

<table>
<thead>
<tr>
<th>Virus Isolate ID#</th>
<th>Code</th>
<th>Genbank Accession No.</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGC (Prototype)</td>
<td>D2-NG</td>
<td>AF 038403</td>
<td>New Guinea</td>
<td>1944</td>
</tr>
<tr>
<td>P7-863</td>
<td>P7-863</td>
<td>U 89517</td>
<td>Malaysia</td>
<td></td>
</tr>
<tr>
<td>ThNH-P36/93</td>
<td>ThNH36</td>
<td>AF 0022441</td>
<td>Thailand</td>
<td>1993</td>
</tr>
<tr>
<td>ThNH-P14/93</td>
<td>ThNH16</td>
<td>AF 0022440</td>
<td>Thailand</td>
<td>1993</td>
</tr>
<tr>
<td>ThNH-P14/93</td>
<td>ThNH14</td>
<td>AF 0022439</td>
<td>Thailand</td>
<td>1993</td>
</tr>
<tr>
<td>ARAC-8110827</td>
<td>D2-JAM</td>
<td>M 15075</td>
<td>Jamaica</td>
<td>1982</td>
</tr>
<tr>
<td>Cook Island-1</td>
<td>COOK-1</td>
<td>AF 004002</td>
<td>Cook Islands</td>
<td>1997</td>
</tr>
<tr>
<td>P7-843</td>
<td>P7-843</td>
<td>U 89518</td>
<td>Malaysia</td>
<td></td>
</tr>
<tr>
<td>S-9730</td>
<td>SEY-8730</td>
<td>M 32952</td>
<td>Seychelles</td>
<td>1977</td>
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<tr>
<td>S-10</td>
<td>10-SOMAL</td>
<td>L 10051</td>
<td>Somalia</td>
<td>1984</td>
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<tr>
<td>Torres Strait-1</td>
<td>TORRES</td>
<td>AF 004019</td>
<td>Torres Strait</td>
<td>1996</td>
</tr>
<tr>
<td>P9-122</td>
<td>P9-122</td>
<td>L 10043</td>
<td>India</td>
<td>1957</td>
</tr>
</tbody>
</table>

A further comparison of alignable amino acid fragments by using neighbour-joining analysis-generated phylogenetic trees (Fig. 2) suggests that DEN-2 strains may be divided into five genotypes with little relationship between their positions in dendrogram and the date of virus isolation. Genotype I is represented by the prototype strain D2-NG; Genotype II includes 1993 isolates from Thailand; Genotype III constitutes the Jamaican strain of 1981 (D2-Jam) and a Malaysian strain P7-863 from an infected Aedes aegypti; Genotype IV includes all the strains from the 1996 Delhi epidemic, Seychelles, Somalia, Cook Islands, Torres Strait, and Malaysian strain P7-843 from infected mosquito; Genotype V includes the older Indian DEN-2 strains from the 1957 and 1967 epidemics. The genetic relationships were independent of the type of the distance estimation programme and the phylogenetic analysis algorithms used (UPGMA; data not shown).
Figure 1. Predicted* amino acid sequence alignment of envelope protein gene and nonstructural protein 1 gene junction (E-NS1) region of 10 dengue 2 (DEN-2) isolates from Delhi with the prototype DEN-2 strain, New Guinea C (D2-NG, genotype I). Corresponding nucleotide sequences have been deposited with the Genbank (see Table 1 for accession #).

*Predicted amino acid sequences of selected global DEN-2 strains (see Table 2 for details), obtained from Genbank, were included for comparison. Dashes (-) indicate identities and dots indicate information not available. Amino acid positions are numbered according to Hahn et al (30) with prototype DEN-2 strain NGC as reference. Single letter amino acid abbreviations: a, alanine; c, cysteine; d, aspartic acid; e, glutamic acid; f, phenylalanine; g, glycine; h, histidine; i, isoleucine; k, lysine; l, leucine; m, methionine; p, proline; q, glutamine; r, arginine; s, serine; t, threonine; v, valine; w, tryptophane; y, tyrosine.
Discussion

The dengue virus infection is an important emerging infection. The incidence of DF/DHF is increasing worldwide and is appearing in areas where it was previously unreported. Though DF has been frequently reported from Delhi since its first appearance in 1967, no major outbreak of DHF had ever been reported until 1996. Natural field isolates from the 1996 Delhi DHF epidemic have allowed us to determine their evolutionary origin.

In the present study, the predicted amino acid sequence data of E-NS1 region of DEN-2 isolates were phylogenetically analysed. The tree thus generated (Fig. 2) revealed that DEN-2 strains isolated from India in 1957 and 1967 probably represented the circulating genotype during that period (Genotype V) and apparently had been evolving independently. In 1996, these strains seem to have been replaced by DEN-2 strains which were quite similar to the strains isolated from Seychelles in 1977 (strain 8730). These strains belonged to the same genotype (Genotype IV).

It has been recently suggested that the introduction of an imported strain (new genotype) into an area of high dengue activity replacing an earlier circulating strain (genotype) may result in a severe form of DEN infection (10). On the basis of the analysis of nucleotide sequence data, Rico-Hesse and her colleagues (13) observed that the South-East Asian DEN-2 strains of two different genotypes were responsible for DHF cases in the Americas, displacing the native American DEN-2 strains which had been circulating earlier in that part of the world. Similarly, in Sri Lanka the introduction of Genotype IV strains in early
1980s replacing Genotype I strains which had been maintained there for 24 years resulted in DHF epidemic\(^{(8)}\). Also, DEN-2 isolates from the 1981 Cuban epidemic of DHF, which were found to be similar to the contemporaneous Jamaican isolates circulating in the Caribbean Islands during that period, replaced the earlier DEN-1 strains in Cuba\(^{(7, 15)}\).

The cause(s) of the more severe form of dengue infection is (are) not fully understood, though it has been postulated that DHF may result from immune enhancement caused by infection by a second DEN serotype\(^{(5)}\). Halstead\(^{(16)}\) also proposed that there are biotypes of DEN that cause DHF at a high rate when they infect permissive hosts. Similarly, Rosen\(^{(6)}\) suggested that more severe forms of the disease may be associated with specific strains which may vary in virulence. However, a direct association between specific genotypes and severity of the disease has not been established.

References

Clinical Features of Hospitalized Patients During Dengue-3 Epidemic in Far North Queensland, 1997-1999

By

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Abstract

Between December 1997 and March 1999, the Cairns and Mossman/Port Douglas regions of Far North Queensland, Australia, experienced an epidemic caused by DEN-3 virus. There were 496 confirmed cases over this period, 98 (20%) of this group were hospitalized. Additionally, 19 patients with probable dengue were hospitalized. The unusually high rate of hospitalization prompted an analysis of the clinical features of the hospitalized cases. The case records of 100 locally-acquired acute dengue cases hospitalized in the local regions were retrospectively examined. The mean age of the hospitalized cases was 42.5 years. In many cases admission was required for the management of severe rash, pain, or dehydration. Gastrointestinal symptoms were common. Other presentations included nephrotic syndrome, hepatitis and encephalopathy. One paediatric case of dengue haemorrhagic fever was recorded. The severity of illness observed in this epidemic is likely to be related to the virulence of the dengue strain.

Key words: Dengue fever, Dengue haemorrhagic fever, IgM IgG Elisa, Australia

Introduction

The city of Cairns (population 111,000) and the two adjacent towns (situated 60 km north of Cairns) of Port Douglas and Mossman (combined population 14,600) are two of the major population centres of Far North Queensland, Australia. The region is...
located in the wet tropical part of Australia and is a popular tourist destination. There are regular flights into Cairns from Papua New Guinea and South-East Asia.

Dengue fever is not endemic in north Queensland but the area is vulnerable to the introduction of the dengue virus. The city of Cairns experienced a major epidemic caused by DEN-1 in 1981-82 and a small epidemic caused by DEN-2 in 1996-97\(^1,2\).

In November 1997, a case of dengue fever, later identified as being caused by DEN-3, was recognized as being acquired in Cairns. A major epidemic ensued, moving briskly through a number of Cairns suburbs. The number of the notified cases fell during the cooler dry season (July-September) only to increase again as the temperature increased in the following summer, when the epidemic moved north to the Port Douglas/Mossman region. The last case of dengue fever during the epidemic was recorded in March 1999. Approximately 220 notifications of dengue fever were received from Cairns and 270 notifications from Port Douglas/Mossman. (Full details of this outbreak are to be presented elsewhere.)

Two hospitals provide inpatient services in the city of Cairns and there is one hospital in Mossman. Of the 496 notified cases, 98 (20%) were hospitalized and a general observation was made that the clinical manifestations of dengue fever during this epidemic were unusually severe. A retrospective analysis of the hospitalized cases was undertaken to record the clinical features.

**Methods**

Diagnostic laboratories or clinicians notified cases of dengue fever to the Tropical Public Health Unit. The cases were classified as 'probable' on the basis of a positive enzyme immunoassay test for IgM antibodies. Classification as definite cases required viral detection by either nucleic acid amplification or viral culture, or positive IgM antibodies against dengue as assessed by haemagglutination inhibition assay (HAI) on fractionated serum. Only the definite cases were notified. At the time of notification, instances of hospitalization were recorded.

Case records of all the notified cases admitted to any of the three hospitals in the region were analyzed. Additional hospitalized cases were included if there was probable dengue. Information extracted from the case notes included age, gender, reason for hospital admission, whether dengue fever was suspected as the cause of illness at the time of admission, the clinical features recorded during admission and laboratory data. Cases were classified as secondary infections if there was prior evidence of dengue virus infection (if the serum IgG ELISA was positive prior to the appearance of IgM), or primary (if IgM reactivity occurred first). If both IgM and IgG were reactive at the time of the initial serum collection, the cases could not be classified as either primary or secondary infections.

Statistics were performed on the tabulated data with ‘instat’ and ‘Epi info, Version 6.04’ programs.
Clinical Features of Hospitalized Patients During Dengue-3 Epidemic in Queensland, 1997-99

Results

A total of 117 hospitalized dengue fever cases were identified, 98 (84%) of which were confirmed. One hundred were admitted to the three hospitals in Far North Queensland, the remainder being hospitalized elsewhere.

Of the 100 patients, 35 acquired their illness in Cairns and 65 in the Port Douglas/Mossman region. The mean age of the patients was 42.5 (range 1 - 76) years. Seven (7%) were aged under 17 years and 13 (13%) were aged above 66 years. Forty-three (43%) of the hospitalized cases were male. The mean duration of hospital stay was 5.4 (range 1-44) days. The mean duration of symptoms prior to admission was 5 (range 1-35) days.

An admission diagnosis of dengue fever was made in 71 of the cases and viral illness in 11. In these cases admission to hospital was deemed necessary for complications such as dehydration or for symptom control. Other admission diagnoses included chest infection or asthma (5 cases), meningitis, encephalitis or psychosis (3 cases), gastro-enteritis (2 cases), pseudomembranous colitis (2 cases), and urinary tract infection (3 cases).

The symptoms recorded in the hospitalized patients are given in Table 1. Fever was documented in most cases. Eleven per cent had a maximum temperature of ≥40°C and 85% had a maximum temperature of ≥38°C. Patients who did not have a fever documented had been symptomatic 3-12 days prior to admission and it is likely that most had been febrile during that period.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Number/Number recorded (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (≥38°C)</td>
<td>85/100 (85)</td>
</tr>
<tr>
<td>Musculoskeletal ache</td>
<td>89/90 (99)</td>
</tr>
<tr>
<td>Headache</td>
<td>80/84 (95)</td>
</tr>
<tr>
<td>Rash</td>
<td>57/87 (66)</td>
</tr>
<tr>
<td>Any gastrointestinal symptoms</td>
<td>84/88 (95)</td>
</tr>
<tr>
<td>Nausea</td>
<td>81/88 (92)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>48/74 (65)</td>
</tr>
<tr>
<td>Altered taste</td>
<td>38/41 (93)</td>
</tr>
<tr>
<td>Ocular pain</td>
<td>38/45 (84)</td>
</tr>
<tr>
<td>Photophobia</td>
<td>10/22 (45)</td>
</tr>
<tr>
<td>Cough</td>
<td>23/47 (49)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>19/30 (63)</td>
</tr>
<tr>
<td>Pruritis</td>
<td>20/23 (87)</td>
</tr>
</tbody>
</table>

Headache and musculoskeletal pain were, as expected, very common, but gastrointestinal symptoms, especially nausea, were also very common. The presence or absence of other symptoms was recorded in less than half of the case records. Where recorded, the presence of altered taste, ocular pain and pruritis were common. Bleeding of any severity was noted in 19 patients. It is likely that for a dramatic symptom such as bleeding, occurrence would be reported but for a symptom such as altered taste under-reporting was likely.

Complications that were ascribed to dengue infection included ‘dehydration’ in 77 patients. The dehydration was, in most cases, not associated with evidence of
haemoconcentration or disturbed renal function. Treatment with intravenous fluids was often commenced as a consequence of nausea.

Rash was described as being particularly severe in 19 patients, with florid petechiae, frank haemorrhage and/or intense pruritis. Bleeding was listed as a complication for 13 of the patients. Amongst these patients the bleeding varied in severity from haematemesis or melaena to haemorrhagic conjunctivitis and vaginal blood loss. For 5 patients ‘collapse’ precipitated their admission. Eight patients had neurological symptoms that varied in severity from mild confusion and drowsiness to features suggestive of encephalitis. All patients made a full recovery.

There were 2 cases that fulfilled the World Health Organization criteria for dengue haemorrhagic fever (DHF)\(^3\). One case occurred in a child with haematemesis and melaena. The serological profile was that of a primary dengue infection. The other, an elderly man with a serological profile of secondary dengue infection, developed nephrotic syndrome.

Overall 80% of the hospitalized patients had a white cell count (WCC) <4 x 10\(^9\)/L (normal range (NR) 4-11 x 10\(^9\)/L); the lowest recorded WCC was 1.0 x 10\(^9\)/L. The neutrophil count was <2.0 x 10\(^9\)/L (NR 2-8 x 10\(^9\)/L) in 72% of the patients. Eighty-two percent of the patients were lymphopaenic (NR 1-4 x 10\(^9\)/L) and 81% had a thrombocytopaenia (NR, 140-400 x 10\(^9\)/L). The lowest recorded platelet count was 8 x 10\(^9\)/L.

Ninety-three patients had measurements of hepatic transaminases and 90% of these patients had levels which exceeded the laboratory normal range for aspartate transaminase (AST) (NR <40U/L). Alanine transaminase (ALT) (NR <45U/L) was elevated in 77% and gamma glutamyltransferase (GGT) (NR <50U/L) was elevated in 60% of the 93 patients. AST was elevated in all of the patients with elevated hepatic transaminases, and three patients had AST levels that exceeded 1000 U/L.

Urinalysis results were recorded for 87 of the patients; blood was detected in 31% and protein in 74%. One patient (mentioned above) had clinical features of the nephrotic syndrome; urinary protein was quantified at 10.8 grams/24 hours.

Creatine kinase levels were measured in 15 patients; six patients had elevated plasma creatine kinase levels (NR <200 U/L).

ELISA was performed on serum from 99 patients and was positive in 85 patients. RT-PCR was performed on serum from 75 patients and was positive in 56. Viral culture was performed on serum from 49 patients and was positive in 22. For serum samples subjected to both viral culture and PCR testing, viral culture detected less than half of the PCR positive samples. There were, however, examples where viral culture was positive and PCR was negative. Three patients had serum without detectable IgM on ELISA, but detectable using HAI of the IgM fraction.
Fifty-five patients had probable primary dengue and 21 probable secondary dengue. Patients with secondary infections were older (p<0.01) and were less likely (p<0.01) to have a rash associated with their illness. The duration of stay in hospital (p>0.05) and other manifestations of illness, including the incidence of haemorrhagic phenomena (p>0.05), were similar in the two groups (Table 2).

Table 2. Clinical and laboratory features of patients with primary and secondary infections

<table>
<thead>
<tr>
<th></th>
<th>Primary infection (n=55)</th>
<th>Secondary infection (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>37.8 (1.5-71) years</td>
<td>51.7 (17-75) years</td>
<td>0.004</td>
</tr>
<tr>
<td>Duration of admission (range)</td>
<td>4.9 (1-13) days</td>
<td>7.0 (2-44) days</td>
<td>0.526</td>
</tr>
<tr>
<td>Rash (%)</td>
<td>39 (71%)</td>
<td>6 (29%)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Bleeding (%)</td>
<td>11 (20%)</td>
<td>5 (24%)</td>
<td>0.762</td>
</tr>
</tbody>
</table>

Discussion

Severe clinical manifestations with a high hospitalization rate characterized the 1997/99 DEN-3 epidemic in the Cairns and Port Douglas/Mossman region of Far North Queensland. Previous epidemics in north Queensland have been associated with much lower hospitalization rates; 12% of the people with dengue fever were estimated to have been hospitalized during an epidemic in the city of Charters Towers in 19934.

Particular features of the epidemic included a very high incidence of gastrointestinal manifestations, and a high incidence of unusually severe rash, in those patients who were hospitalized. In most other regards the clinical features were typical of dengue fever. The apparently low reported rates of altered taste and pruritis are possibly a reflection on the retrospective nature of the study. A number of the hospital doctors were unfamiliar with the minor clinical features of dengue fever and did not enquire routinely about the presence of symptoms such as taste perversion and skin itch.

One case of DHF was documented in a child. This was the first case of paediatric DHF recorded in Australia since the early part of the 20th century. The serological profile in this case was consistent with a primary dengue infection.

One case of nephrotic syndrome was associated with dengue fever. This is an unusual complication of dengue fever. Immune complex deposition in renal tissue has been occasionally reported5. The high prevalence of urinary abnormalities in our study suggests that renal involvement is common in acute dengue although abnormal renal function itself is rare.

Liver involvement in the hospitalized cohort was also common. The AST was elevated more often than the ALT, which is a pattern that has been observed previously6. Hepatic disorders, in conjunction with bleeding and thrombocytopenia, have been proposed as an indicator of severe disease7. Several patients had a clinical picture dominated by their hepatitis.

The high prevalence of elevated CK levels in the small group actually tested suggests that myositis was common in this outbreak. This is no great surprise considering the prominent musculo-skeletal symptoms in classical acute dengue fever.
dengue. Histological changes have been reported in muscle biopsies in acute dengue\textsuperscript{8,9} and clinical cases of dengue myositis have been reported\textsuperscript{10}.

Patients with secondary dengue infection were older than those with primary infection. This is likely to have been the result of the previous major epidemics in the region that occurred ≥18 years ago\textsuperscript{2}.

Primary and secondary dengue infections are currently classified according to serum antibody titres determined by haemagglutination inhibition assays. This test was not routinely performed and with the increasing use of ELISA worldwide it will become more difficult to estimate the proportion of dengue fever cases that are primary or secondary. The criteria used during this epidemic are readily assessable for many patients. Semi-quantitative determination of IgG levels is possible using immunochromatographic card tests and provides additional clues for the detection of secondary infection\textsuperscript{11}. A need exists to modify the criteria for primary and secondary infections that take the evolving patterns of testing into account.

There have now been three major epidemics of dengue fever in north Queensland in recent years; Townsville/Charters Towers in 1992/93 (DEN-2), Torres Strait Islands in 1996/97 (DEN-2) and this most recent epidemic in Cairns and Port Douglas/Mossman. All of these epidemics have occurred in populations with a limited previous exposure to dengue infections. The severity of an average dengue illness is difficult to measure. The clinical impression that the dengue illness seen in this epidemic was more severe than in the other dengue epidemics was supported by the high rate of hospital admission. This supports the hypothesis that some dengue strains are intrinsically more virulent than others.

References
Dengue and Dengue Haemorrhagic Fever and its Control in Maldives

By
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Abstract
Maldives recorded DHF for the first time in 1979, and again in 1983. The first serious outbreak occurred in 1988. During this epidemic 2054 cases with all types of manifestations, viz. DF, DHF and DSS, were registered and nine children <10 years of age died. The 1998 and 1999 outbreaks occurred after a gap of 10 years with 1750 and 1835 cases, respectively. For the first time, using the PanBio diagnostic kits, 81 DF and 15 DHF cases and 59 DF and 38 DHF cases were detected during 1998 and 1999, respectively. One child died in May 1999. The outbreak was controlled by a three-pronged attack in an integrated approach, using space spray, larvicidal application with temephos and community participation with the active involvement of schoolchildren, which was coordinated by the Ministry of Health. No cases were recorded in the port/airport areas because of an effective vector control programme.

Key words: DF/DHF, Aedes aegypti, Community participation, Maldives

Introduction
Maldives is an archipelago in the Indian ocean with a population of about 250,000 people spread over 1190 small coral islands varying in size from 0.25 sq km to 10 sq km. These islands are located about 200 km south-west of India. Most of the islands are at low level elevation from the sea line ranging from 1-5 metres and are devoid of any stream or river. Only 202 islands are inhabited and the source of fresh water supply is from underground aquifor and rain water collections.

The rainfall average is between 1600-1900 mm and is contributed by the south-west (May-August) and north-east (October-January) monsoons. The climate is tropical hot and humid, with temperatures ranging between 25°-31°C and humidity between 70%-90% throughout the year.

Emergence of DF/DHF in Maldives
Till mid 1970s, the DHF epidemic was localized in several south-east Asian
countries. Thereafter there was a dramatic expansion westwards, and by 1980, DHF had taken India, Sri Lanka and Maldives into its orbit.

**DHF outbreak, 1998 and 1999**

Maldives recorded its first outbreak of DF/DHF in 1979 and one thereafter in 1983. The 1988 outbreak was the worst so far when 2054 cases were registered and 9 children (below the age of 10 years) died. All the three types of dengue, i.e. DF, DHF and DSS, were reported. After a gap of 10 years, another outbreak occurred in 1998 when a total of 1750 cases were reported with no death. For the first time, using a series of diagnostic kits from PanBio Pty. Ltd., Australia, the Indira Gandhi Memorial Hospital (IGMH) in Malé, confirmed 81 DF and 15 DHF cases. In 1999, as of September, there were 59 DF and 38 DHF cases, with 1 death. The majority of the cases (78/97=80.4%) occurred during March-June 1999 just before and during the north-west monsoon period (Table 1). In 1999 (up to end of March), out of the 52 DF and 26 DHF cases reported from IGMH, the majority of the cases (34/52=65.4%) and (23/26=88.5%) occurred, respectively, in children less than 10 years of age. It is surprising that for both the DF and DHF infections in Maldivian children, males suffered 2.7 times and 2.3 times more than females respectively and there is no significant difference in the proportion of infected males suffering from DF and DHF infections ($\chi^2=0.13$, p=0.72). DEN-1 and DEN-2 were identified to circulate in the country.

### Table 1. DF and DHF cases during 1998 and 1999 in Malé town in Maldives

<table>
<thead>
<tr>
<th>Month</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>DHF</td>
</tr>
<tr>
<td>January</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td>June</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>July</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>August</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>September</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>October</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>15</td>
</tr>
</tbody>
</table>

**Vectors**

Both Aedes aegypti and Aedes albopictus have been recorded in Maldives. Practically, all the islands have recorded the presence of both the species. However, Aedes aegypti is confined to domestic environment, while Aedes albopictus is most prevalent in peripheral areas, breeding quite frequently in tree holes.

In a study undertaken in Malé town and other atolls during September 1999, it was brought out that the number of positive containers in key premises ranged from 3 to 8 positives containers while the number of larvae in key containers ranged from approximately 500 to 10,000. The key containers detected were rainwater tanks, cemented construction areas and agricultural
pits. The high-risk areas included schools, mosques and residential areas occupied by expatriates.

**Control of outbreak**

Maldives does not have a regular dengue control programme. In the event of an outbreak vector control activities are undertaken by malaria and other vector-borne disease control project (Mal & VBDC). For the control of the outbreak in 1998-99, three campaigns of two-week duration each were undertaken during May 1998, December 1998 and May 1999. The control activities included: space spraying using malathion 95% technical premium grade ULV formulation at a dose of 10 litres per 0.25 km of space. This was effected by motorized knapsack sprayers filled with a special nozzle for ULV spraying.

**Larviciding with temephos 500 EC**

Environmental sanitation and source reduction through community participation with active coordination between the Ministry of Health and Ministry of Education. Large numbers of schoolchildren took part in this activity.

Personal protection. People were encouraged to use all types of repellents (mats, coils, mosquito creams) to prevent mosquito bites.

**Vector control at port/airport**

Vector surveillance and control activities at the port/airport areas were undertaken regularly by the staff of Malaria Control Programme conforming to international health regulations to keep these areas free of mosquito vector breeding. These activities included source reduction, larviciding with temephos and space spraying of passenger areas and fumigation of storage godowns.

**Capacity-building**

To improve the skills of staff and give them hands-on experience, two workshops (in May and September 1999) of 3-day duration each on entomological techniques and vector control practices were organized at Malé. Participants were drawn from VBDC, and airport/seaport organizations. Emphasis was laid on the identification of breeding habitats of vectors of malaria, filariasis and dengue and elimination of the same through source reduction and chemical methods under field conditions.

**Intersectoral coordination**

To promote intersectoral coordination and community participation, a 3-day workshop was organized in September 1999 at Laamu Atoll. Forty participants comprised of family health workers, teachers and island women development committees participated in the workshop. They were familiarized with mosquito surveillance techniques and their control through source reduction and use of fish in a partnership approach.

**References**


Sero-Diagnosis of Dengue Infections by Haemagglutination Inhibition Test (HI) in Suspected Cases in Chittagong, Bangladesh

By M.M.M.Amin, A.M.Z. Hussain, M.Murshed, I.A. Chowdhury, S. Mannan, S.A. Chowdhuri, and D. Banu
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Abstract
A hospital-based cross sectoral serological study was undertaken in Chittagong metropolitan city - the second largest city in Bangladesh - to assess the current receptivity of the country for DF/DHF, using the haemagglutination inhibition test (HI). Samples were taken from the suspected cases of dengue infection following the inclusion and exclusion criteria. A total of 253 paired samples was collected from selected children aged between 1-15 years. A total of 18 (7.1%) samples were interpreted as positive ones out of which 9 (50.0%) were male children and the remaining 9 female children. Primary dengue infection was serologically diagnosed in 4 patients. Seven samples (male 4, female 3) produced results which were suggestive of definite secondary dengue infection while 1 male sample was interpreted as either primary or secondary dengue infection, and 6 (3 male, 3 female) samples were interpreted as presumed secondary infection. Five to 9.9-year-old children were the most vulnerable group as 10 (55.6%) out of a total of 18 positive samples came from this group. The monthly distribution of positive cases showed that December 1996 topped the list with 5 (27.8%) cases, followed by November 1996 and October 1996 with 4 (22.2%) positive samples each.

Key words: Dengue fever, Dengue haemorrhagic fever, Haemagglutination Inhibition Test, Bangladesh

Introduction
Dengue fever/dengue haemorrhagic fever (DF/DHF) caused by DEN-1, DEN-2, DEN-3 and DEN-4 is widespread in countries of South-East Asia(1). In Bangladesh, during 1965, there was an outbreak of dengue infection called ‘Dhaka fever’, which was the first documented outbreak of DF(2). After this outbreak, blood samples collected from the Medical College, Dhaka, were identified as of DEN-3, probably
the first such cases identified in the Indian subcontinent\(^3\). A few cases of DEN-1 and DEN-2 were also found in 1977-1978 in selected areas of Bangladesh\(^4\). In 1982-83, the Institute of Epidemiology, Disease Control & Research, Mohakhali, Dhaka, conducted a survey in which 278 samples out of the 2465 samples taken from a number of schools in Dhaka city were found positive with DEN-1 infection. During the period 1984-86, three samples out of 21 collected from Mitford Hospital and Shisu Hospital, Dhaka, were found to be positive by the haemagglutination inhibition test (HI). It was believed that the low prevalence of the infection was due to low vector densities in cities. Since DF/DHF was not only showing a geographical spread but also greater frequency, a serodiagnostic study was designed during September 1996-June 1997 using the HI test to assess the proportion of DF/DHF amongst viral febrile patients attending the Chittagong Medical College Hospital and other epidemiological factors.

**Methodology**

**Study design, population and sample size**

This was a hospital-based descriptive cross-sectional survey. The survey defined and identified eligible patients suspected of dengue infections. A total of 253 paired blood samples from the selected cases attending the outpatient and inpatient departments of the Chittagong Medical College Hospital were collected. The study population comprised individuals aged between 1-15 years. The study population was selected according to the following inclusion and exclusion criteria set exclusively for the survey.

**Case inclusion criteria**

Febrile illness for 72 hours, 1-15 years of age, no focal clinical signs within next 48 hours, negative for any other infections, based-up chest X-ray, complete blood, malarial parasite, urine routine examination.

**Case exclusion criteria**

If routine lab tests and clinical features during the 48 hours between admission/attendance and the time of collection of blood sample for dengue serology suggested other infections, the case was excluded. During revisit, in case of outpatients, if the patient was lost and/or refused to give blood sample, then the case was excluded and the corresponding first samples were discarded if the patient or the guardian refused to participate in the survey.

**Blood collection**

First blood samples were drawn from suspected dengue cases on admission into hospital or attendance at the outdoor clinic. Second blood samples were collected just prior to the discharge in case of admitted patients. In case of outpatients, blood samples were collected after one week following the first sample.

**Results**

A total of 253 paired samples were collected from the selected children aged between 1-15 years, with a mean age of 7.11 years. Male children constituted the main bulk (155,
61.3%) and female children numbered 98 (38.7%). The five to 9.9-year-old children group constituted the bulk of the respondents (55.7% of the total). Fifty-eight children (22.9%) were from the 10-15-year-old age group and 54 from the 1-4.9-year-old age group. Eighteen (7.1%) samples were interpreted to be positive ones, while 18 samples were interpreted as 'not dengue' infection, although they showed raised titre but no change in the antibody response in the convalescent sera.

Analysis by sex and type of infection

Out of the 18 positive samples, 9 were male and 9 were female children. Primary dengue infection was serologically diagnosed in 4 patients while 7 children suffered from a definite secondary infection, 1 from definite infection, possible primary or secondary, and 6 from presumed secondary infection (Table 1).

By age group and frequency of infection

When analysed according to age group, we found that 5-9.9-year-old children were the most vulnerable group as 10 (55.6%) out of a total of 18 HI-positive patients came from this group. The next most vulnerable group was the 1-4.9-year-old children. A total of 6 (33.3%) children from this group suffered from dengue infection while the least affected group was the 10-15-years-olds (Table 2).

By age group and type of infection

Table 3 shows that younger children dominated the $2^0$ and $4^0$ types of infection. Five children out of 6, in the 1-4.9-year-old age group suffered from these two types of secondary dengue infections while 6 out of 10 from the 5-9.9-year-old age group were diagnosed to have either $2^0$ or $4^0$ type of infection.
Distribution by month

The month-wise distribution of positive cases showed that December 1996 topped the list with 5 (27.8%) cases out of the total 18 serologically positive cases, followed by November 1996 and February 1997 with 4 positive cases each (Table 4).

Table 4. Month-wise distribution of serologically positive cases

<table>
<thead>
<tr>
<th>Month</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>Total HAI positive cases</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 1996</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Oct 1996</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td>Nov 1996</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td>Dec 1996</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Mar 1997</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Apr 1997</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

Distribution by season

The seasonal occurrence of positive samples showed that the post-monsoon period was the most affected period (13 cases, 72.22%) ahead of the monsoon period (3 cases, 16.7%), while the pre-monsoon period which has been found in other countries to be the contracting peak time for contracting dengue virus through mosquito bites, accounted for only 2 (11.11%) sero-positive cases (Table 5).

Table 5. Seasonal occurrence of dengue infection according to positive samples

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of positive samples</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-monsoon (January-March)</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Monsoon (April-September)</td>
<td>3</td>
<td>16.67</td>
</tr>
<tr>
<td>Post-monsoon (October-December)</td>
<td>13</td>
<td>72.22</td>
</tr>
</tbody>
</table>

Discussion

The interpretation was based on the WHO criteria set for HI. The positive results included primary, secondary and primary or secondary, and presumed secondary infections. The results and discussion were mainly based on the positive serological outcome, with negative, not dengue and uninterpretable results, ignored. A total number of 18 (7.1%) samples out of 253 paired samples tested by HI techniques were found to be positive. Given the general conception, even among physicians, that Bangladesh was free from the clutches of dengue, the positive findings of 7.1% among the suspected cases should be a warning signal for physicians and policymakers as well. It is worrisome that 13 samples were interpreted as definite infection, secondary or presumed infection, secondary infection (Table 1) that gives rise to the possibility of severe forms of dengue cases in the future and also demonstrates the endemicity of dengue in Bangladesh. In spite of the large number of secondary type of dengue infection, not a single patient manifested dengue shock syndrome (DSS) that is expected more often to occur as we see in other south-east Asian countries.
The most vulnerable age group was 5-9.9-year-old children with 55.6% infection followed by the 1-4.9-year-old age group (33.3%). This may be due to the fact that children in these two age groups remain largely in their homes and become vulnerable to the bites of the vector.

It has been found elsewhere in the world that the pre-monsoon season is the most favourable period when dengue infection takes place. But in this study, 72.22% of the total serologically positive samples were collected in the post-monsoon period spanning from October to December. Pre-monsoon and monsoon periods were found to be less favourable (Table 5). The reasons behind this deviation need to be unearthed and understood. But one thing we must recognize that samples were collected at a higher rate in these three months, i.e. October - 30 samples (11.9%), November - 33 samples (13.0%) and December - 39 samples (15.4%). During these three months alone, 40.3% of the total 253 samples were collected.

The results of this study suggest that there is no room for complacency as regards dengue infection. Bangladesh can experience the devastating onslaught of dengue infection at any time as all the factors favourable for such an outbreak are present in all the major cities of the country.

Acknowledgements
We acknowledge with thanks the contributions of Mr Matin and Mr Md. Ali, Laboratory Technicians, and other staff of the Virology Department, IEDCR, which helped immensely to complete this study.

References
6. Dengue Haemorrhagic Fever (DHF); DHF situation and activities in the WHO South-East Asia Region in 1988. WHO Wkly Epidemiol Rec 1989, 64:175-76.
Introduction

In the last few years, dengue and dengue haemorrhagic fever have been recognized as important emerging infectious diseases in the tropics and subtropics around the globe\(^1\). There are four dengue serotypes, viz. DEN-1, DEN-2, DEN-3 and DEN-4, in circulation worldwide. Persons living in dengue endemic areas are at risk of four dengue infections during their lifetime. Some characteristics of the vector, the virus and the host have been identified as risk factors for the development of the severe disease, i.e. dengue haemorrhagic fever (DHF), but the most important of all is the sequential infection\(^2,\)\(^3\).

DHF epidemics of 1981 and 1997 in Cuba

Cuba suffered an extensive epidemic of classical dengue in 1977-78 caused by DEN-1 (not a single case of DHF). This was followed by a DHF outbreak in 1981, caused by...
DEN-2. The 1981 epidemic was the first and the most severe DHF epidemic ever to be recorded in the American region. No DHF or fatal cases were observed in children 1 and 2 years of age. These children were born after the 1977-1978 epidemic and hence was the only group in the Cuban population that did not suffer a secondary infection\(^{(4)}\). Secondary infections were demonstrated in almost 98-99% of individuals (children and adults) with DHF\(^{(5,6)}\).

After the epidemic was controlled, a campaign to eradicate *Aedes aegypti* from Cuba was launched. Extensive environmental and chemical measures succeeded in reducing the house index (% houses infested by *Ae.aegypti*) from well above 10.9% at the beginning of the intensive control operations in August 1981 to 0.007% by April 1984\(^{(7)}\). The degree of control of vectors in Cuba was apparently high enough to interrupt all transmission of dengue. There was no viral activity between 1982 and 1996 in the entire country as evidenced by an effective surveillance system for this disease.

In January 1997, a DEN-2 outbreak was detected in Santiago de Cuba municipality (in an eastern Cuban province). Several risk factors for the re-emergence of dengue were identified. These were: high vector infestation, increased migration of people from endemic countries to the municipality, limited water supply, deficiencies in solid waste disposal, and inadequate vector control activities in the municipality.

A total of 17 259 febrile cases were initially considered to be dengue suspected cases; however, serological studies confirmed the infection in 3012 individuals. Only DEN-2 was recovered by viral isolation on C636 cell line and PCR from the patients studied. Of the serologically confirmed dengue cases there were 205 DHF cases with 12 fatalities\(^{(8,9)}\). All these DHF cases were classified according to the Guideline for Prevention and Control of Dengue and Dengue Haemorrhagic Fever in the Americas\(^{(10)}\). Fever (100%), haemorrhagic manifestations (100%), headache (91.2%), abdominal pain (86%) and myalgia (75.1%) were observed in the 205 DHF/DSS (dengue shock syndrome) patients. Thrombocytopenia and shock were observed in 99% and 15.1% of the cases respectively, and 100% of the cases presented haemconcentration. Around 68.7% and 30.2% of the cases presented ascites and pleural effusion. Shock was observed in the 12 fatal cases. 51.2% of cases had hepatomegaly and 38% vomits. The youngest case was 17-year-old and the oldest 66 years. One hundred and two (102) patients were female (49.7%).

The epidemic was completely controlled by August 1997 although the last case was reported in November 1997. May and June were the months with the highest incidence. The vector control measures arrested the spread of the disease to other parts of the country and the epidemic was confined to this municipality only. (Cuba had at that moment 30 out of 169 municipalities where *Aedes aegypti* was present.)

The Cuban experience is probably unique. In two different epidemics, 16 years apart, which occurred in an immunologically defined population, there was a clear
demonstration that second dengue infections were the most important risk factors leading to DHF. Yet another epidemiological observation revealed that in the 1997 epidemic, DHF cases were observed only in adults. Only one DHF case occurred in an individual younger than 16 years of age. In Cuba, only adults were at risk of a secondary dengue infection because dengue transmission was interrupted in 1981. Correspondingly, all children were at risk of a primary dengue infection. Part of the adult population of the Santiago de Cuba municipality was immune to DEN-1 and therefore at risk of secondary DEN-2 infection. Serological studies demonstrated secondary IgG antibody response in 91.6% (11/12) of the fatal cases and in 98.2% of the 113 DHF studied cases\(^9,11\). One interesting and new observation was the possibility that DHF could still occur in an individual who had acquired a secondary infection almost 20 years following a primary infection. To our knowledge, this kind of finding has not been reported before. Data from a dengue surveillance system plus the known low vector mosquito density rates demonstrated that no dengue viruses had circulated in Cuba between 1982-1996 as no flavivirus IgG antibodies were detected in sera from children studied in the dengue surveillance system. From 1989 to 1996, more than 9000 paired sera obtained from persons with febrile illness were tested and no dengue cases were confirmed.

We must emphasize the high severity of both the 1981 and 1997 Cuban epidemics when compared with some others which occurred in the American region. However, when both the epidemics in Cuba are compared, the severity of the disease during the 1997 epidemic is more pronounced. The case-fatality rate (CFR) during the 1997 epidemic was 0.40/100 DF cases and 5.8/100 DHF/DSS cases. On the contrary, the CFR during the 1981 epidemic was 0.046/100 DF cases and 1.5/100 DHF/DSS cases. The rate fatality of 16-year-interval DHF infections (1997 epidemic) was higher than for the 4-year-interval secondary infections (1981 epidemic), suggesting thereby that the risk for DHF persists for many years (perhaps for life) after the first dengue infection; which means that all individuals with previous dengue infections should be aware of the increased risk of DHF when they travel to dengue-endemic areas. Of course, other risk factors for DHF such as race, chronic diseases, the types of viruses that produce both the first and second infections, and, even more important, the genetic characteristics of these viruses, can modulate this risk. In the Cuban experience, in both the epidemics, the sequence of infections was the same, DEN-1 followed by DEN-2. Although the genotype of the second virus was different, both had their origin in Asia\(^8,12,13\).

Finally, another interesting observation that could explain the high severity of these epidemics was the ethnic groups of the people involved. During the 1981 DHF epidemic, white people were considered a risk factor for the severe form of the disease. DHF/DSS, both among children and adults, was significantly higher among the Whites (p<0.01)\(^14\). In the second epidemic, in 1997, once again, Whites were a risk factor for the severe form of the disease. Of the total 169 DHF/DSS cases reported, 46.7% were Whites, 35.5% Mulattos and only 16% Blacks. Racially, the population ratio in
DHF Epidemics in Cuba, 1981 and 1997: Some Interesting Observations

Santiago de Cuba is 30.1% Whites, 42.8% Mulatto and 26.8% Black. Among the 12 fatal cases, two were Blacks (16.6%).

Since 1997, no dengue circulation has been documented on the island. A strong entomological and seroepidemiological surveillance system has been established and only imported dengue cases have been reported. The campaign to control and eradicate Aedes aegypti is being maintained. Finally, we want to record that the 1997 epidemic which occurred in a municipality of 475,580 inhabitants was thoroughly studied. Clinical records were reviewed by experts. The table 1 includes key observations from this outbreak.

Table 1. Summary of the main observations of dengue in Cuba (1977-97)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cases</td>
<td>500 000</td>
<td>344 203</td>
<td>3 012</td>
</tr>
<tr>
<td>DHF/DSS cases</td>
<td>None</td>
<td>10 312</td>
<td>205</td>
</tr>
<tr>
<td>Fatalities</td>
<td>None</td>
<td>158 (101 children)</td>
<td>12 (adults)</td>
</tr>
<tr>
<td>Viral agent</td>
<td>DEN-1</td>
<td>DEN-2</td>
<td>DEN-2</td>
</tr>
<tr>
<td>Genotype</td>
<td>American</td>
<td>South Asia</td>
<td>South Asia</td>
</tr>
<tr>
<td>Population at risk of secondary infection</td>
<td>-</td>
<td>44.46%*</td>
<td>18.5%**</td>
</tr>
<tr>
<td>Interval of infection</td>
<td>-</td>
<td>4 years</td>
<td>16-20 years</td>
</tr>
</tbody>
</table>

* Of the Cuban population
** Of the Santiago de Cuba population

Finally, we want to call the attention of the international community to the fact that it is possible to control dengue when the principles established by PAHO/WHO for the purpose are strictly followed. Cuba has done it twice.

References


Predictive Indicators for Forecasting Epidemic of Dengue/Dengue Haemorrhagic Fever Through Epidemiological, Virological and Entomological Surveillance

By

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Abstract
Dengue/dengue haemorrhagic fever is the leading cause of death from the declared infectious diseases in children in southern Vietnam. The dengue epidemic in 1998 was an extensive one, with the morbidity rate of 455 cases/100 000 pop. and mortality rate of 1.27/100 000 pop. DEN-3 was the predominant serotype. However, in 1999, there was a lower morbidity (80.7 cases/100 000) and a lower mortality (0.23 deaths/100 000). The circulation of DEN-3 virus decreased and that of DEN-4 virus emerged. In both the years, most of the cases and deaths occurred in children under 15 years of age. Shock accounted for 15.3% and 13.5%, respectively, of all DF/DHF cases in 1998 and 1999, of which 2.5% and 2.1% died. The epidemic curve reached its peak in the rainy season (June to October).

An excessive increase in the number of cases compared with the average of the previous five years and an increased circulation of a new dengue virus serotype during the first quarter may be used as indicators to predict larger epidemics in the year. There was no correlation of entomological indices with the epidemiological situation in this study.

Key words: Dengue fever, Dengue haemorrhagic fever, Epidemics, Vietnam

Introduction
Dengue fever/dengue haemorrhagic fever (DF/DHF) continues to be a major public health problem in Vietnam, especially in the southern region. In the southern region, with a population of over 27 million and high temperatures throughout the year, DF/DHF is the leading cause of high mortality in children from the declared infectious diseases. The annual DF/DHF morbidity rates fluctuate from 100 to 450 cases/100 000 pop. and the number of deaths ranges from 60 to 220 per year.

In 1998, an extensive DHF epidemic occurred in southern Vietnam ever since its first appearance in 1963. A total of 123 997 cases (accounting for 52% of all country's cases) and 347 deaths (accounting for 83%
of all country’s deaths) were reported. In 1999, the DHF prevalence and number of deaths decreased remarkably.

Throughout the two consecutive epidemics of DF/DHF, an assessment was made of the epidemiological, virological and entomological surveillance indicators that may be used to predict large outbreaks early enough to mount an effective control programme. An analysis of the same is presented in this paper.

Methods and materials

Epidemiological surveillance

The number of cases and deaths were reported from hospitals according to the clinical and laboratory criteria prescribed by the World Health Organization (2). The current surveillance system was used for data collection, reporting and feedback. Data of cases and deaths were reported weekly or monthly from hospitals to district preventive medicine centres and then to the Provincial Preventive Medicine Centre. From here, these data were sent to the Pasteur Institute, Ho Chi Minh City, and finally to the Ministry of Health.

Serological and virological surveillance

Virus isolation was done at the Pasteur Institute by using C6/36 cells (Aedes albopictus) with blood samples collected from acute DF/DHF patients. Specific IgM antibody of dengue was detected in patients’ sera by using Mac-ELISA tests. Serum samples were collected from the patients at least 5 days after the onset of the illness.

Entomological surveillance

The adult mosquito density index and Breteau index were used in the study.

Results

Morbidity and mortality

Table 1 includes the DF/DHF cases and deaths reported during the years 1993-1999. It is apparent that the number of cases and deaths in 1998 were two times more than the average during the previous five years (1993-1997) (455 cases compared with 217 cases and 1.27 deaths as compared with 0.62 deaths) while in 1999, the number of cases and deaths were less than a half of the average reported during 1993-1997. However, the case-fatality rate showed a decreasing trend from 1993 onwards (from 0.41% to 0.28%). This was possibly due to improvements in the clinical management of DF/DHF cases.

Seasonal variation

Figures 1 and 2 show the distribution of morbidity and mortality in 1993-1997, 1998 and 1999. DF/DHF cases occurred throughout the year, but the peak season was from June to October which is the rainy season in Vietnam. From the figures, we can identify that during the early months of 1998, the epidemic curve was much higher than that of 1993-1997 (the average number during the previous five years). From this, one could predict a large DF/DHF epidemic occurring in 1998. In comparison, during the early months of 1999, one could predict that there would be no big epidemic in this year.
Table 1. Number of DF/DHF cases and deaths reported from 1993 to 1999

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases</th>
<th>Cases/ 100,000 pop.</th>
<th>No. of deaths</th>
<th>Deaths/ 100,000 pop.</th>
<th>CFR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>43048</td>
<td>172.7</td>
<td>177</td>
<td>0.71</td>
<td>0.41</td>
</tr>
<tr>
<td>1994</td>
<td>25901</td>
<td>104.1</td>
<td>61</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>1995</td>
<td>51897</td>
<td>203.7</td>
<td>165</td>
<td>0.65</td>
<td>0.32</td>
</tr>
<tr>
<td>1996</td>
<td>55974</td>
<td>288.0</td>
<td>178</td>
<td>0.67</td>
<td>0.32</td>
</tr>
<tr>
<td>1997</td>
<td>77370</td>
<td>319.9</td>
<td>222</td>
<td>0.83</td>
<td>0.29</td>
</tr>
<tr>
<td>Average of 1993-1997</td>
<td>50838</td>
<td>217.7</td>
<td>160.60</td>
<td>0.62</td>
<td>0.31</td>
</tr>
<tr>
<td>1998</td>
<td>123997</td>
<td>455.7</td>
<td>347</td>
<td>1.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Decrease in 1998 over 1993-97</td>
<td>234.9%</td>
<td>209.3%</td>
<td>216%</td>
<td>204.2%</td>
<td>90.3%</td>
</tr>
<tr>
<td>1999</td>
<td>21951</td>
<td>80.7</td>
<td>65</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>Increase in 1999 over 1993-97</td>
<td>43.2%</td>
<td>37.1%</td>
<td>40.5%</td>
<td>37%</td>
<td>93.6%</td>
</tr>
</tbody>
</table>

Figure 1. Monthly distribution of reported DF/DHF cases in Southern Vietnam, 1993-1997, 1998 and 1999
Age distribution and disease severity

DF/DHF cases in 1998 and 1999 occurred mostly in children under 15 years of age (i.e. 90% of the total cases).

Shock accounted for 15.3% and 13.5% of all cases of DF/DHF in 1998 and 1999 respectively, while 2.5% and 2.1% of the shock cases died.

Virus isolation

Table 2 shows the virus isolation rate distributed by month in 1998 and 1999. Most of the dengue viruses were isolated during June - October. The mean positive isolation rate was 11.6% in 1998 and 10% in 1999. In 1998, even in January and February, the isolation rates were high, being 18.9% and 10.6% respectively. DEN-3, the new serotype which had emerged since 1994, was the predominant serotype. So, at that time, a large epidemic was predicted to occur in that year.

Figure 3 shows the correlation of the dengue serotypes isolated with the number of DHF cases reported during 1993-1999. The positive isolation rate of DEN-3 increased gradually from 1994 to 1997 (from 1% to 4%), and got its peak (8.2%) among other serotypes in 1998, resulting in the largest epidemic this year (455 cases/100 000 pop). In 1999, the circulation of DEN-3 was lower: 3.6%. DEN-1 continued to decrease from 1.4% in 1998 to 1% in 1999. DEN-2 increased from 1.7% (1998) to 3.4% (1999). Surprisingly, DEN-4 emerged in 1997, and increased gradually with a positive rate of 0.32% in 1998 and 2% in 1999. The emergence of this serotype may predict a large epidemic breaking out in the near future.
Table 2. Monthly distribution of positive virus isolation rate in 1998 and 1999

<table>
<thead>
<tr>
<th>Year</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 (1.4)</td>
</tr>
<tr>
<td>D1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17 (1.4)</td>
</tr>
<tr>
<td>D2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>21 (1.7)</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>18</td>
<td>17</td>
<td>19</td>
<td>2</td>
<td>15</td>
<td>4</td>
<td>0</td>
<td>101 (8.2)</td>
</tr>
<tr>
<td>D4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>4 (0.32)</td>
</tr>
<tr>
<td>Positive tests/ tests</td>
<td>17/90</td>
<td>15/142</td>
<td>0/10</td>
<td>3/57</td>
<td>5/103</td>
<td>25/244</td>
<td>25/179</td>
<td>25/104</td>
<td>2/78</td>
<td>20/161</td>
<td>6/153</td>
<td>0/104</td>
<td>143/1236</td>
</tr>
<tr>
<td>%</td>
<td>18.9</td>
<td>10.6</td>
<td>0</td>
<td>5.3</td>
<td>4.9</td>
<td>10.2</td>
<td>14.0</td>
<td>24.0</td>
<td>2.6</td>
<td>12.4</td>
<td>11.3</td>
<td>0</td>
<td>11.6</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 (1)</td>
</tr>
<tr>
<td>D1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6 (1)</td>
</tr>
<tr>
<td>D2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>20 (3.4)</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>12 (2)</td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>12 (2)</td>
<td></td>
</tr>
<tr>
<td>Positive tests/ tests</td>
<td>0/37</td>
<td>0/21</td>
<td>1/35</td>
<td>1/34</td>
<td>2/37</td>
<td>8/46</td>
<td>8/64</td>
<td>13/65</td>
<td>5/47</td>
<td>7/70</td>
<td>11/56</td>
<td>3/74</td>
<td>59/586</td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>0</td>
<td>2.85</td>
<td>3.94</td>
<td>5.4</td>
<td>17.39</td>
<td>12.5</td>
<td>20.00</td>
<td>10.63</td>
<td>10.0</td>
<td>19.64</td>
<td>4.05</td>
<td>10.07</td>
</tr>
</tbody>
</table>

Figure 3. Correlation of positive dengue virus isolation rate with DF/DHF morbidity in Southern Vietnam, 1993-1999
Serological surveillance

The IgM antibody anti-dengue was identified from the patients’ sera in all months of the years 1998 and 1999. The mean seropositive rate was over 50% in 1998 and 44.3% in 1999. The seropositive result of Mac-ELISA tests during the pre-epidemic stage (first quarter) may predict a large epidemic occurring during the succeeding months of the year.

Entomological data

Breteau and density indices at sentinel surveillance points in Southern Vietnam for the period 1993-97, 1998 & 1999 are included in Figures 4 and 5.

It is evident from Figures 4 and 5 that there was no correlation between the entomological data and the epidemiological situation: the curves of A. aegypti density index and Breteau index of 1998 (the year of the largest DHF epidemic) were lower than that of the years 1993-1997. However, the curve of 1998 was sometimes higher than that of 1999.

Conclusion

There was a large DHF epidemic in 1998 in southern Vietnam, with high numbers of cases
and deaths; and DEN-3 was the predominant serotype. In 1999, there was a great remission of DHF cases and deaths, but there was a resurgence of DEN-4, a new serotype, which has tended to increase gradually. Any emerging new serotype may be used as a predictive indicator.

The excessive increase in the number of DHF cases and deaths in the first quarter of 1998 was noted to compare with the average morbidity of the previous five years. At the same time, there was an increased circulation of the new serotype of dengue virus and the seropositive results of Mac-ELISA tests in the pre-epidemic stage, all these may be considered as predictive indicators for a larger DHF epidemic.

There was no correlation between the entomological indices and the epidemiological situation in this study.

References
Use of Permethrin-treated Curtains for Control of Aedes aegypti, in the Philippines

By
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ABSTRACT
An experimental study on the use of permethrin-treated curtains for the control of mosquito vectors for dengue was conducted in two barangays in Cebu city. These two barangays are highly endemic for dengue fever and are fairly comparable in relation to their populations and economic levels. Sixty-five houses in Barangay Labangon were included in the study group while 65 houses in Barangay Mabolo were included in the control group. Treatment of the curtains in the study group was done after an entomological survey had been conducted in the area at the start of the study. No chemical intervention was done in the control group after the initial survey.

Entomological surveys were carried out in both barangays for six months in order to determine the changes in the vector density levels. Results of the surveys showed that there was a drop in the mosquito larval indices in both groups, although a significant and greater percentage of drop was noted in the study group.

The results of the study showed the effectiveness of the use of permethrin-treated curtains as a vector control measure. This strategy should be given due consideration as one of the major approaches in eliminating the DF/DHF vectors.

Key words: Aedes aegypti, Permethrin, Curtains, Philippines

Introduction
DHF is one of the mosquito-borne viral diseases that is increasingly becoming a major public health problem. Based on a ten-year average of morbidity and mortality rates, Region 7 in the Philippines is one of the three regions with the highest number of dengue fever cases in the country. Within Region 7, the highest number of cases are found in the densely populated Cebu city.\(^1\)
Since the disease is transmitted through the bite of the mosquito, strategies for its prevention are focused on integrated vector control. During emergency control, fogging is undertaken; however, the effect is short lived. Thus, the use of permethrin-treated curtains as a form of chemical control in high vector density areas has been resorted to in order to find out if this strategy can provide an effective and long-lasting strategy for vector control\(^2,^3\). The studies were initiated in August 1996 and concluded in May 1997.

### Methods and materials

#### Selection of study areas

The study included two barangays, namely, Labangon and Mabolo, which were highly endemic for dengue fever in Cebu city. Labangon was selected as an experimental group while Mabolo was taken as the control group. The two sample sites were chosen on the basis of comparability with regard to the number of dengue fever cases, their populations and socioeconomic status of the inhabitants. The sample households were selected by systematic sampling. The distance between the two barangays was more than 200 metres, the normal flight range of the *Aedes* mosquito. Only households having 2-3 curtains made of light cotton material were included. The sample size was computed following a study undertaken in Vietnam\(^2\) on the use of permethrin-treated bamboo curtains for dengue vector control.

Basic data on the socio-demographic profile was collected through structured interviews using the entomological questionnaires. The actual larval collection was done in August, at the start of the rainy season, in 65 sample households each in both the barangays. After the introduction of treated curtains in the experimental barangay, larval collection was followed by the entomological teams who were accompanied by barangay officials and health workers in the sample households monthly for the next five months. During the larval collection, actual destruction of breeding places of mosquitoes was carried out in both barangays.

#### Treatment of curtains

The chemical used in the study was Coopex 25% EC which contains 250 g/litre of the residual pyrethroid, permethrin. Curtains of the 65 sample households in the experimental barangay were soaked in 25% EC permethrin at a 1:20 dilution (1 litre of the chemical to 20 litres of water). Twenty-one litres of the solution was enough to soak 80-100 pieces of curtains made of light cotton material. Two large basins were used, one for soaking the curtains and the other for catching the excess solution from the soaked curtains. After soaking the curtains for at least 2 minutes, they were allowed to drip in the other basin for a few minutes so that the excess solution from the drippings could still be used. The curtains were then air-dried and were not exposed to direct sunlight.

#### Results

Before the introduction of the treated nets, both sample barangays revealed very high House, Container and Breteau indices, indicating that the vector density was high in...
both areas (Tables 1 and 2). After intervention, the indices in both barangays dropped but a greater percentage of decrease was noted in Labangon where treated nets had been introduced. The average decrease of House Index in Labangon was 36.4% as compared to the average decrease of 24.2% in Mabolo. For the Container Index and the Breteau Index, the average decrease in Labangon was 12.4% and 77% respectively, while in Mabolo, the average decrease of these indices was 7.7% and 51.3% respectively. Tests for the level of significance (two-tailed test) in the differences of the percentage decreases in both barangays showed that there was a significant difference in the decrease of the indices between Labangon and Mabolo.

Table 1. Larval indices by month, Labangon – Exp. Group - (1996-1997)

<table>
<thead>
<tr>
<th>Barangay</th>
<th>Months</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labangon</td>
<td>No. of houses inspected</td>
<td>65</td>
<td>66</td>
<td>61</td>
<td>64</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>No. of houses (+) for larvae</td>
<td>46</td>
<td>31</td>
<td>19</td>
<td>23</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>No. of containers inspected</td>
<td>382</td>
<td>452</td>
<td>428</td>
<td>325</td>
<td>270</td>
<td>314</td>
</tr>
<tr>
<td></td>
<td>No. of containers (+) for larvae</td>
<td>85</td>
<td>52</td>
<td>33</td>
<td>36</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>House Index</td>
<td>70.7</td>
<td>51.3</td>
<td>31</td>
<td>35</td>
<td>25.4</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Container Index</td>
<td>22.3</td>
<td>11.5</td>
<td>7.7</td>
<td>11</td>
<td>9.3</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>Breteau Index</td>
<td>131</td>
<td>78.8</td>
<td>54</td>
<td>56</td>
<td>33.3</td>
<td>46</td>
</tr>
</tbody>
</table>

The most common indoor containers in Labangon found as the breeding sites of the Aedes mosquitoes were jugs, bottles, plastic water containers, drums, flower vases and jars, while the most common outdoor containers positive for the larvae were used tyres, drums, plastic water containers and tin cans.

In Mabolo, the most common indoor breeding sites of the Aedes larvae were flower vases, drums and water tanks. For outdoor containers, the most common were tyres, tin cans, pails, plastic water containers and drums. Among the natural containers, bamboo stumps were the most common breeding sites in both areas.

Table 2. Larval indices by month, Mabolo - Control Group - (1996-1997)

<table>
<thead>
<tr>
<th>Barangay</th>
<th>Months</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mabolo</td>
<td>No. of houses inspected</td>
<td>65</td>
<td>63</td>
<td>67</td>
<td>64</td>
<td>65</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>No. of houses (+) for larvae</td>
<td>34</td>
<td>22</td>
<td>25</td>
<td>12</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>No. of containers inspected</td>
<td>389</td>
<td>411</td>
<td>436</td>
<td>321</td>
<td>215</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>No. of containers (+) for larvae</td>
<td>64</td>
<td>45</td>
<td>45</td>
<td>16</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>House Index</td>
<td>52.3</td>
<td>34.9</td>
<td>37</td>
<td>18.7</td>
<td>21.5</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>Container Index</td>
<td>16.5</td>
<td>10.9</td>
<td>10</td>
<td>4.9</td>
<td>10.2</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Breteau Index</td>
<td>98.5</td>
<td>71.4</td>
<td>67</td>
<td>25</td>
<td>33.8</td>
<td>38.8</td>
</tr>
</tbody>
</table>
Discussion

Both the experimental and control barangays initially were comparable in almost all aspects like population, household size, average family income, household characteristics, total number of dengue cases and even vector density. Both barangays demonstrated very high vector densities indicating that both areas were at risk of having outbreaks based on the WHO density figure. The comparability of both areas was necessary to control factors that may affect the results of the study.

After intervention, a drop in the mosquito density in both areas was noted because of health education and actual elimination of breeding places conducted during the entomological survey. However, a more significant decrease was noted in the barangay where permethrin-treated curtains were used, signifying the effectivity of the use of such curtains for dengue vector control. In addition, use of these curtains is relatively safe as no side-effects were observed among the household members.

In the fourth month, a slight increase in the indices in Labangon was noted due to the fact that some households had started to change and wash their curtains at this time. In the sixth month, a slight increase in the indices was again noted. By this time, more than 60% of the households had already washed their curtains at least once and 52% of the households were not using their curtains any more.

The breeding places commonly observed in the study areas were plastic water containers or jugs, water storage drums, used tyres, flower vases and tin cans. It is essential to take note of the containers or breeding places of the mosquitoes so that health education and environmental sanitation activities will be focused on these breeding places. Knowing the breeding places would indirectly reveal the attitude and practices of the community with regard to water storage, garbage disposal and environmental sanitation.

Conclusion

Two significant activities in the prevention and control of dengue emerged in this study. First, it is important to do an entomological survey to determine the kind of containers mosquitoes prefer to breed in and the vector density in an area before instituting any intervention. Knowledge about preferred containers as breeding places will aid the health worker in conducting the information, education and communication (IEC) campaign against dengue fever.

Treatment of curtains with permethrin proved to be an effective vector control measure against dengue fever. This strategy, together with health education and environmental sanitation, should be considered when planning for a comprehensive dengue prevention and control programme.

References

Aedes aegypti, Dengue and Re-urbanization of Yellow Fever in Brazil and other South American Countries - Past and Present Situation and Future Perspectives

By

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ABSTRACT

Dengue (DEN) and yellow fever (YF) viruses are two important arboviruses causing human disease. Dengue fever and dengue haemorrhagic fever (DF/DHF) reemerged in the Americas after Aedes aegypti had reinfested most tropical and subtropical regions in the hemisphere. The number of DF/DHF cases being reported are increasing each year; and in South America only Chile and Uruguay have not reported any cases. Sylvan YF has increased in the last two decades because of increasing human contact with forest areas, and the risk of its re-urbanization has increased dangerously due to the presence of Aedes aegypti proximally to areas with sylvatic YF, particularly in Bolivia, Brazil, Colombia and Peru, where YF is transmitted by Haemagogus mosquitoes. This paper gives an overview of the main epidemiological findings of YF and DEN viruses in the recent past and discusses the future perspectives of the dissemination of DF/DHF and re-urbanization of YF in South America. Current situation demands the adoption of a massive YF vaccination programme in the region in conjunction with a powerful regional plan of Aedes aegypti control to avoid deaths attributed to these two viruses in South America.

Key words: Dengue/Dengue haemorrhagic fever, Yellow Fever, Brazil, South America, Aedes aegypti

* Address for correspondence: Dr Pedro Fernando da Costa Vasconcelos, Instituto Evandro Chagas, Av. Almirante Barroso, 492, 66090-000, Belém, PA Brazil. Fax (005591) 226-1284/226-5262. Email – pedrovasconcelos@iec.pa.gov.br
Introduction

For many decades, a programme for the eradication of *Aedes aegypti* was carried out in countries of the continental America. The programme was planned and initiated by the Pan American Health Organization (PAHO) in 1946. Initially the principal aim was the prevention of urban epidemics of yellow fever (YF) virus and of dengue epidemics. During the first 30 years, substantial progress was made and several countries were able to eradicate this mosquito. Unfortunately, some countries, which included Argentina, French Guyana, the United States, Venezuela and several Caribbean countries, failed to achieve eradication. Some countries abandoned the programme, as in the case of Venezuela where transmission of dengue virus was still occurring when the government terminated its efforts in this direction\(^{(1)}\). Several Latin American countries stayed *Aedes aegypti*-free from 1961 to 1974, but slowly and gradually these countries were re-infested from the ones where the problem had remained unresolved. The resurgence of this mosquito was soon accompanied by the occurrence of dengue fever (DF) and dengue haemorrhagic fever (DHF) epidemics. It also resulted in an increased risk of the re-urbanization of the YF virus, an important viral agent responsible for large epidemics in the region in the past with high case-fatality rates. This article reviews the past and present situation of these two important arboviruses causing human disease and their vectors, and analyses the future perspectives.

Past situation

DF/DHF

In the Americas, the first documented DF epidemic occurred in 1779-80 in the city of Philadelphia, U.S.A., which was clinically characterized as “breakbone fever”\(^{(2)}\). The incidences of DF epidemics in the continental America were reported in the Caribbean region, affecting several countries, as well as in southern United States during the 18\(^{th}\) century, at least on four occasions\(^{(3)}\).

During the first half of that century, more DF epidemics occurred in the same countries, as also in Cuba, Mexico, Panama and several other Caribbean islands. The number of people that got affected was virtually unknown, but have been estimated to be in thousands. The clinical signs and symptoms observed included fever, headache, muscle and small-joint pains\(^{(3,4)}\).

In South America, the occurrence of DF epidemic was registered in Brazil during 1846-1848 and 1851-1853, and in this century in 1916 and 1923\(^{(5,6)}\). Neutralizing antibodies to DEN-1 and DEN-2 were found in inhabitants more than 50 years of age in several municipalities of the Amazon Valley, which suggested the circulation of these serotypes in that region of Brazil in the first years of the twentieth century\(^{(7)}\). Following these episodes, a new DF epidemic was reported in 1981-1982, in Boa Vista, state of Roraima, in the Amazon region at the border of Guyana and Venezuela, the countries that were suffering DF epidemics. This was the first time that dengue have been diagnosed using specific laboratory tests, and was isolated from human beings and mosquito vectors in Brazil \(^{(8)}\).

Other South American countries that experienced DF epidemics in this century were Peru during the 1950s and Venezuela between 1941-1946\(^{(9)}\).
Venezuela reported DF epidemics during the 1960s, the period when almost all South American countries had eradicated Aedes aegypti. DEN-2 and DEN-3 were responsible for the transmission\(^4{,}\,10\).

During the 1970s DEN-2 and DEN-3 were responsible for DF epidemics in Colombia, a country that had achieved eradication of the vector during the PAHO programme. Transmission also occurred in Guyana, French Guyana, Surinam and Venezuela\(^9\).

During the 1980s the expansion of dengue accompanied the distribution of the vector, Aedes aegypti, and during these years, a second potential vector, the Asian mosquito, Aedes albopictus, was introduced in the region\(^11\). New countries reported DF epidemics, which included Bolivia, Ecuador, Paraguay, Peru and Brazil. These countries had not experienced DF epidemics before or dengue viruses were absent from there for many years or decades. The extended new circulation of dengue viruses, the susceptibility of almost all inhabitants and the high rates of vector mosquito indices, resulted in large-scale dengue transmission with explosive epidemics.

In Brazil that had more than 130 million inhabitants and had serious problems related to water supplies and waste management in urban areas, the spread of dengue viruses was quick. In 1986, when Rio de Janeiro state, south-eastern region, was infected with DEN-1\(^12\), in the same year this serotype was responsible for the epidemics in Ceara and Alagoas states in the north-eastern region. Three years later, eight states in three geographical regions reported DF epidemics (Table 1). Tens of thousands of cases were notified, but a survey carried out among schoolchildren in the metropolitan area of Rio de Janeiro city estimated the occurrence of about one million infections during 1986-1987\(^13\).

During these DF epidemics in Brazil and other Latin American countries, a few cases of DHF were also reported. Except the 1981 Cuban DHF epidemic, no other DHF epidemic was reported in the continental America\(^10,\,14,\,15,\,16,\,17\).

In the years 1989-1990 an epidemic of DHF was reported throughout Venezuela, which was the second major DHF epidemic in the Americas. It reported over 6,000 cases and 73 deaths. DEN-1, DEN-2 and DEN-4 were isolated during the episode, but only DEN-2 was recovered from four fatal cases\(^4,\,9\).

**Yellow fever (YF)**

The YF virus was one of the most important human infectious diseases in the past, and a model in epidemiology and public health. YF was first suspected to be associated with Aedes aegypti by the Cuban scientist, Carlos Finlay, and its transmission was established by the US Major, Walter Reed. Before and after the works of Finlay and Reed, the virus was responsible for devastating epidemics in urban centres of Africa and America\(^18,\,19,\,20\). Fortunately, the discovery of the YF vaccine by Theiler & Smith\(^21\), a milestone in medical history, was responsible for the prevention of the YF urban epidemics. Effectiveness of the vaccine was so high that
Table 1. Dengue cases reported to Ministry of Health, Brazil, by geographical region, 1982-1998

<table>
<thead>
<tr>
<th>Year</th>
<th>Countrywide</th>
<th>North</th>
<th>Northern</th>
<th>C. West</th>
<th>Southeast</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>12,000</td>
<td>12,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1986</td>
<td>47,370</td>
<td>-</td>
<td>13,802</td>
<td>-</td>
<td>33,568</td>
<td>-</td>
</tr>
<tr>
<td>1987</td>
<td>89,394</td>
<td>-</td>
<td>28,479</td>
<td>-</td>
<td>60,915</td>
<td>-</td>
</tr>
<tr>
<td>1988</td>
<td>190</td>
<td>-</td>
<td>120</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>1989</td>
<td>5,334</td>
<td>-</td>
<td>4,213</td>
<td>-</td>
<td>1,121</td>
<td>-</td>
</tr>
<tr>
<td>1990</td>
<td>40,642</td>
<td>-</td>
<td>15,950</td>
<td>1,606</td>
<td>23,086</td>
<td>-</td>
</tr>
<tr>
<td>1991</td>
<td>97,209</td>
<td>2,194</td>
<td>8,020</td>
<td>4,346</td>
<td>82,649</td>
<td>-</td>
</tr>
<tr>
<td>1992</td>
<td>3,215</td>
<td>-</td>
<td>396</td>
<td>1,671</td>
<td>1,148</td>
<td>-</td>
</tr>
<tr>
<td>1993</td>
<td>7,086</td>
<td>-</td>
<td>788</td>
<td>1,462</td>
<td>4,836</td>
<td>-</td>
</tr>
<tr>
<td>1994</td>
<td>56,621</td>
<td>18</td>
<td>49,828</td>
<td>5,864</td>
<td>911</td>
<td>-</td>
</tr>
<tr>
<td>1995</td>
<td>132,180</td>
<td>3,221</td>
<td>57,974</td>
<td>32,819</td>
<td>35,111</td>
<td>3,055</td>
</tr>
<tr>
<td>1996</td>
<td>179,780</td>
<td>2,788</td>
<td>125,400</td>
<td>14,839</td>
<td>32,230</td>
<td>4,523</td>
</tr>
<tr>
<td>1997</td>
<td>254,939</td>
<td>22,174</td>
<td>196,203</td>
<td>12,962</td>
<td>22,879</td>
<td>721</td>
</tr>
<tr>
<td>1998</td>
<td>536,398</td>
<td>36,669</td>
<td>248,943</td>
<td>17,415</td>
<td>230,704</td>
<td>2,667</td>
</tr>
<tr>
<td>Total</td>
<td>1,462,358</td>
<td>79,064</td>
<td>750,116</td>
<td>92,984</td>
<td>529,228</td>
<td>10,966</td>
</tr>
</tbody>
</table>

Source: FUNASA, Ministry of Health (Brazil).

In continental South America the last cases of urban YF were reported in 1942\(^5,17,19\). In spite of the existence of the jungle maintenance cycle of the virus\(^22\), the vaccine could reduce the impact of YF sylvatic outbreaks, and contributed decisively to the arrest of its re-urbanization. On the other hand, the vector eradication programme developed under PAHO leadership also maintained the Aedes aegypti-free status of many countries that had been free from YF epidemics.

During the ‘60s, ‘70s and ‘80s, YF cases or outbreaks were reported almost exclusively in Bolivia, Brazil, Colombia and Peru. The vectors responsible for the transmission were mosquitoes of the forest canopy, chiefly Haemagogus spp. and to lesser extent Sabethes chloropterus, and possibly Aedes fulvus in Brazil\(^19,23,24,25\).

In Brazil, excepting the three epidemics which occurred in Goiás, Pará and Maranhão states\(^17,26,27\), sporadic cases or small outbreaks were documented.

**Present situation**

**DF/DHF**

Epidemics of DF have exploded in the American region. To date, except Chile and Uruguay, all countries have reported DF epidemics and at least five of them - Brazil, Colombia, Ecuador, French Guyana and Venezuela - have suffered major or minor DHF epidemics\(^4,10\). In 1998, Argentina reported for the first time in its history an epidemic of DF after 82 years\(^28\), and Uruguay became infested with Aedes aegypti, after more than 30 years of freedom.
from it. Therefore, presently, only Chile is
Aedes aegypti-free\(^{10}\).

In Brazil, Aedes aegypti has quickly
spread over the whole country during the
current decade. To date, all the 27 states are
infested by the vector, and transmission has
been reported in at least 23 states in all the
five regions (Table 1 and Figure 1). In 1990,
DEN-2 invaded Rio de Janeiro\(^{29}\), and an
epidemic during 1990-1991 resulted in the
reporting of 99,707 DF and 462 DHF cases
and eight deaths\(^{30}\). In 1991, this serotype
was responsible for the first epidemic of DF
in Tocantins state\(^{31}\) in the Amazon region,
about 2,000 km away from Rio de Janeiro.
And, in 1994, a large epidemic of DF, with
24 cases of DHF with 11 deaths, was
reported in Ceará state\(^{16}\). This serotype, to
date, has been found in at least 16 states,
while DEN-1 has been recognized in 20
states, and co-circulation of both serotypes
reported in at least 12 states. The number of
DF cases has increased dramatically in Brazil
in all its geographical regions since 1994,
especially in the north-east and south-east\(^{30}\).
These two regions reported more than
1,100,000 cases, representing 87.5% of all
notified dengue cases in the country (Table
1, and Figure 1).

**Table 2.** Number of cases and deaths of DHF
reported in the Americas by country, 1981-1998\(^{*}\)

<table>
<thead>
<tr>
<th>Country</th>
<th>DHF cases</th>
<th>Deaths</th>
<th>Case-fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venezuela</td>
<td>34,193</td>
<td>284</td>
<td>0.8</td>
</tr>
<tr>
<td>Colombia</td>
<td>13,512</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>Cuba</td>
<td>10,517</td>
<td>170</td>
<td>1.6</td>
</tr>
<tr>
<td>Mexico</td>
<td>3,391</td>
<td>137</td>
<td>4.0</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>3,141</td>
<td>26</td>
<td>0.8</td>
</tr>
<tr>
<td>Brazil</td>
<td>795</td>
<td>40</td>
<td>5.0</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>546</td>
<td>51</td>
<td>9.3</td>
</tr>
<tr>
<td>Dominican Rep.</td>
<td>446</td>
<td>30</td>
<td>6.7</td>
</tr>
<tr>
<td>El Salvador</td>
<td>289</td>
<td>12</td>
<td>4.1</td>
</tr>
<tr>
<td>Trinidad</td>
<td>182</td>
<td>10</td>
<td>5.5</td>
</tr>
<tr>
<td>Jamaica</td>
<td>108</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Other countries</td>
<td>252</td>
<td>29</td>
<td>11.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>67,372</strong></td>
<td><strong>853</strong></td>
<td><strong>1.3</strong></td>
</tr>
</tbody>
</table>

\(^{*}\)Source: PAHO. Provisional data for 1998.

Venezuela has reported annually hundreds of DHF cases, with dozens of
fatalities. From 1990 till 1998, this country reported to PAHO 34,193 cases of DHF and
284 deaths, and since then has been the
leading country reporting DHF in the region
and in all Americas (Table 2). Next to
Venezuela, so far, DHF cases have occurred
is Colombia, which reported 13,512 cases
and 60 deaths. Brazil has notified 795 DHF
cases and 40 deaths, and, finally, French
Guyana, with 57 DHF cases with two fatal
outcomes\(^{10}\). A comparison of the
occurrence of DF/DHF among Brazil, other
South American countries and North/Central
American countries is given in Table 3.
Table 3. Reported DF/DHF cases/deaths in Brazil, South America and North-Central America in 1990s. Comparison of the percentage for each region*

<table>
<thead>
<tr>
<th>Year</th>
<th>Brazil N(%)</th>
<th>DHF (Deaths)</th>
<th>South America # N(%)</th>
<th>DHF (Deaths)</th>
<th>North-Central America N(%)</th>
<th>DHF (Deaths)</th>
<th>Americas N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>40,642(34.4)</td>
<td>274(8)</td>
<td>38,318(32.4)</td>
<td>3,364(53)</td>
<td>39,265(33.2)</td>
<td>8(1)</td>
<td>118,225</td>
</tr>
<tr>
<td>1991</td>
<td>97,209(61.8)</td>
<td>188(0)</td>
<td>22,678(14.4)</td>
<td>2,076(26)</td>
<td>37,453(23.8)</td>
<td>45(2)</td>
<td>157,340</td>
</tr>
<tr>
<td>1992</td>
<td>3,215(5.3)</td>
<td>-</td>
<td>25,262(42.0)</td>
<td>1,142(17)</td>
<td>31,705(52.7)</td>
<td>611(9)</td>
<td>60,182</td>
</tr>
<tr>
<td>1993</td>
<td>7,086(8.8)</td>
<td>-</td>
<td>44,556(55.3)</td>
<td>3,187(20)</td>
<td>28,935(35.9)</td>
<td>1002(5)</td>
<td>80,577</td>
</tr>
<tr>
<td>1994</td>
<td>56,621(31.3)</td>
<td>24(11)</td>
<td>52,554(29.0)</td>
<td>4,175(14)</td>
<td>71,907(39.7)</td>
<td>537(34)</td>
<td>181,082</td>
</tr>
<tr>
<td>1995</td>
<td>132,180(38.6)</td>
<td>112(2)</td>
<td>92,685(27.0)</td>
<td>6,408(57)</td>
<td>118,095(35.4)</td>
<td>1,708(54)</td>
<td>342,960</td>
</tr>
<tr>
<td>1996</td>
<td>179,780(60.6)</td>
<td>69(1)</td>
<td>53,971(18.2)</td>
<td>3,437(24)</td>
<td>62,820(21.2)</td>
<td>1,586(53)</td>
<td>296,571</td>
</tr>
<tr>
<td>1997</td>
<td>254,939(60.3)</td>
<td>35(5)</td>
<td>63,711(15.1)</td>
<td>10,250(71)</td>
<td>104,178(24.6)</td>
<td>1,498(77)</td>
<td>422,828</td>
</tr>
<tr>
<td>1998†</td>
<td>536,398(72.8)</td>
<td>93(13)</td>
<td>102,062(13.8)</td>
<td>10,896(64)</td>
<td>98,540(13.4)</td>
<td>1,326(14)</td>
<td>737,000</td>
</tr>
<tr>
<td>Total</td>
<td>1,308,070(54.6)</td>
<td>795(40)</td>
<td>495,797(20.7)</td>
<td>44,935(346)</td>
<td>592,898(24.7)</td>
<td>7,419(249)</td>
<td>2,396,765</td>
</tr>
</tbody>
</table>

*Source: Pan American Health Organization; # Excepting cases from Brazil; † Provisional data.

**Yellow fever**

The major vector of YF virus is Haemagogus janthinomys, a species with strict sylvatic habits that it stays all the time in the forest canopy, biting only when people intrude into the forest\(^17,23,27\).

The present occurrence of jungle YF in Latin America has shown widespread distribution over the last decades to four countries (Brazil, Bolivia, Colombia and Peru). In the last two years, besides these four countries, Venezuela and Ecuador have reported some cases that occurred after decades of absence of YF (Figure 2). A couple of cases have also been reported in Surinam (imported from Brazil) and French Guyana.

The number of cases and deaths reported during 1985-1998 in different periods are shown in Figure 3. The case-fatality rate for YF in South America during this period was 53.2%. Compared with DHF (with case-fatality rate of 1.3% in the Americas), the low number of YF cases is not accompanied by low lethality. It will not be surprising that in the event of an urban YF epidemic, the number of deaths would be very high as a consequence of the large number of susceptible populations in almost all countries. The situation could turn out to be more dramatic in countries like Argentina, Chile and Uruguay, where almost all inhabitants are non-immune for YF and, as such, susceptible to the virus. In the past, too, it was observed that any occurrence of the urban transmission of YF was followed by a dramatic increase in mortality, and the degree of risk remains the same\(^17,18,32\) today.
Figure 2. Yellow fever cases reported to PAHO by South American countries, 1970-1998

![Chart showing yellow fever cases reported to PAHO by South American countries (1970-1998)](source: PAHO/WHO)

Figure 3. Yellow fever cases and deaths reported in South America, 1985-1998*

![Chart showing yellow fever cases and deaths reported in South America (1985-1998)](source: PAHO/WHO. Partial data, subject to change)

* Source PAHO/WHO. Partial data, subject to change.
Despite the availability of the YF 17D vaccine that for decades has been used with excellent response (giving protection for at least 10 years), large population groups still remain unimmunized. Many of them live in high-risk areas where transmission is closeby or re-infestation by Aedes aegypti has recently occurred. These areas have been identified in the YF endemic regions of Brazil, Bolivia, Colombia and Peru, the four countries that have reported more of YF cases in the Americas.

**Aedes aegypti susceptibility to YF virus**

Presently, YF cases in South America have only been transmitted by sylvatic vectors, especially *Haemagogus janthinomys*. But the susceptibility of the Aedes aegypti population circulating in Latin America to YF virus needs to be established in order to assess the increased risk of the re-emergence of the urban transmission. It is possible that the annual occurrence of several cases in Brazil and hundreds of cases in Peru and Bolivia might have been transmitted by Aedes aegypti. Surprisingly, urban transmission has not been reported yet, excepting six limited cases in Santa Cruz, Bolivia, but denied by the health authorities of that country.

A warning is coming and the advice is clear: there are two complementary ways to follow; first, the vaccination of all inhabitants in South America, initially for the people living in countries where YF transmission is established through Aedes aegypti, later in regions of DEN focus inside of YF endemic areas, and, finally, in countries with DEN epidemics and in regions outside of YF endemic areas. It is equally essential to establish a powerful Aedes aegypti control programme in South America, or preferably in all Americas, to prevent DHF epidemics.

**Future perspectives**

**Large epidemics of DF/DHF**

It is possible that in a few years, South America will become an endemic region where massive epidemics of DF/DHF will occur affecting millions of people. The number of DF/DHF cases are already on the increase (Table 3). It is known that several factors play a role in DEN transmissibility, but the three most important factors are the following:

The growth of population: The population growth in urban centres in South America has not been accompanied by an improved level of environment. This growth has facilitated the re-establishment of Aedes aegypti and favoured the perpetual breeding of the disease vector. This has been further facilitated by increased migration of people to urban areas which suffer from unplanned and haphazard growth without any civic facilities. Accumulation of solid wastes becomes a difficult proposition to handle. Civic authorities are unable to provide enough drinking water, which results in large-scale storage practices.

Lack of public health services: Because of financial constraints, the governments of the countries have not been able to develop enough public health services which could cope with the problem. There is also a paucity of trained personnel who could manage vector control activities.
Change in human life style: The globalization of world economies has brought in a sea-change in the lifestyle of people. The facilities for fast long-distance travel have facilitated the transportation of viruses in incubation and viremia periods from Africa and Asia to Europe and America and vice versa. This, probably, was the major mechanism which facilitated the spread of dengue viruses in the world. Another factor is the disposable receptacles. These receptacles, in many countries, have been the source of collecting clean water, especially rain water, which not only promoted extensive breeding of the vector species but also resulted in its geographical spread.

Urban transmission of YF

From 1985 to the first half of 1998, PAHO reported 2,603 cases and 1,385 deaths due to sylvatic YF in Latin America (Figure 3), giving a case-fatality rate of 53.2%. These numbers, from our point of view, do not represent the reality of the disease because these figures are hospital-based and do not represent the total infections. It is known that severe forms of YF represent no more than 10% of all infections, and also that the inapparent and mild forms are not diagnosed, except in ongoing studies during epidemics(17). Under-notification is the rule and it is possible that many cases, including severe cases, are not correctly diagnosed. Consequently, these cases are neither notified to government authorities nor to PAHO/WHO, hence no control measures are adopted.

On the other hand, we do not believe that the YF virus has no contact with Aedes aegypti, since the countries reporting YF cases have also reported DF/DHF cases. As in the case of Brazil, high indices of Aedes aegypti have been reported from all over the country. Secondly, in Brazil the vector is also present in the endemic areas of YF and many states have reported DF as well as YF cases too(17).

Therefore, the risk of YF’s re-urbanization is increasing, especially outside the endemic areas where the vaccine coverage is very low. To illustrate, in the first half of 1999 (data not shown), Brazil reported 46 YF cases and some patients moved during incubation and viremia periods to areas reporting DF cases (Vasconcelos PFC, personal information). Logically, a contact of Aedes aegypti with these patients facilitated the transmission. Fortunately, urban YF cycle was not established, although an increased risk will be felt for a long time.

CONCLUSION

The occurrence of DF/DHF epidemics and the increased risk of re-urbanization of YF need a prompt response from the health authorities in the region in order to prevent in the new millennium a picture similar to what was observed in the first decades of the twentieth century, when thousands of lives were lost especially due to urban YF epidemics(19,33).

Consequently, a continental initiative to control the spread of DF/DHF and to prevent the re-urbanization of YF in South American countries is essential. International organizations such as PAHO/WHO, in our view, may take this initiative and urge the
countries of the region to strengthen public health services to meet these heightened challenges. It should be possible to train manpower for the professional management of solid waste disposal, improve drinking water supplies to prevent storages, and develop surveillance mechanisms both for disease vectors and cases with well-equipped hospital services for better management of severe cases.

There is a strong case for establishing linkages between various government departments to promote intersectoral coordination and to develop information, education and communication (IEC) programmes and to involve communities, NGOs and other voluntary agencies in a spirit of partnership.

YF vaccination is yet another area which requires attention. We know 17D vaccine provides protection for 10 years or more but reports are also available which show protection up to 35 years\(^{35,36}\). PAHO/WHO may support more research into this aspect and also help the countries financially for indigenous production of this vaccine for mass vaccination.

Networking of information (possibly through the Internet) is equally important for the dissemination of updates on cases/deaths and other risk factors.

These initiatives, taken collectively by the governments of the countries, and actively supported and facilitated by PAHO/WHO, will go a long way in preventing/controlling DF/DHF and YF epidemics in the continent.

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Key breeding Sites of Dengue Vectors in Hanoi, Vietnam, 1994-1997

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Abstract
From 1994 to 1997, a total of 6,357 water containers in four districts of Hanoi were investigated for the types, capacity, water volume, positive percentage with Aedes aegypti larvae and number of larvae in different instars. Six types of water containers were found as breeding sites of DF/DHF vector, including drums (38.8%), concrete tanks (26.2%), clay jars (26.0%), discarded objects (5.0%), aquariums (3.5%) and buckets (0.5%). Two species, Aedes aegypti and Aedes albopictus, were found from the 47,479 Aedes larvae collected, of which Aedes aegypti were dominant (87.2%). 91.7% of Aedes aegypti larvae were collected from three types of big water containers (drums, tanks and jars). Larval breeding significantly increased during the rainy season from April to November.

Key words: Aedes aegypti, Aedes albopictus, Key containers, Vietnam

Introduction
During the recent past, DF/DHF has not yet become a serious health problem in the urban area of Hanoi, Capital of Vietnam1). However, surveillance of dengue vector population and its breeding sites play a very important role in predicting DF/DHF epidemics and effectively establishing active vector control measures. This study aims at determining the key containers which breed Aedes aegypti and the seasonal change of their population in Hanoi.

Materials and methods
The studies were conducted in four communes of Hanoi city which included: Van Ho 3 alley, Le Dai Hanh commune, Hai Ba Trung district, Hang Be street, Hang Bac commune, Hoan Kiem district, Kham Thien street, Trung Phung commune, Dong Da district, Doi Can street, Doi Can commune, Ba Dinh district. The study lasted for four years, from 1994 to 1997.
Adult mosquito surveys were conducted on indoor resting mosquito collection (2 well-trained technicians, 15'/house, 15 houses/study site, and twice a month at 15-days interval). Water containers were classified by types, capacity, and water volume, and percentage of water containers positive with Aedes aegypti larvae. Aedes larvae were collected by entomological nets and identified in the medical entomology laboratory. Data collected were analysed using EPI INFO 6.0 and EXCEL 7.0 software.

Result and discussion

DF/DHF vector surveillance

From 1994 to 1997, 8 mosquito species comprising of 4 genus were found in Hanoi with the presence of both Aedes aegypti and Aedes albopictus. Aedes aegypti were dominant among the total of collected Aedes mosquitoes. Almost all Aedes albopictus adult mosquitoes were found only at their breeding sites.²,³,⁴

Surveillance results of adult mosquito, larval population and DF/DHF cases (Fig. 1 and Fig. 2) show that there was no relationship between the mosquito density index, the Breteau index and the number of DF/DHF cases ($r=0.288; 0.140$). This means that these indices had very small potential for predicating the DF/DHF epidemic in Hanoi. Data on vector surveillance also revealed that Aedes aegypti population significantly increased during the rainy season from April to November.
Breeding sites

The results of the investigations on water containers (Table 1) showed that the types of the most popular domestic water containers were: tanks (38.9%) (average capacity: 950 litres), clay jars (30.2%) (average capacity: 120 litres), drums (26%) (average capacity: 200 litres), buckets (1.7%) (average capacity: 9.5 litres), discards (1.9%) (average capacity: 0.35 litres) and aquariums (1.2%) (average capacity: 30.5 litres). Aedes aegypti larvae were mainly found in drums (40.35%), jars (28.22%), concrete tanks (20.85%) and discarded objects (8.96%). Meanwhile, Aedes albopictus larvae were mainly found in jars (40.70%), discarded objects (24.45%), drums (16.63%), and aquariums (9.86%). This means that the percentage of each type of water container positive with Aedes aegypti larvae differed from one type to another. Types of containers producing almost all of Aedes larval population were called "key breeding sites". These are drums, jars and tanks in Hanoi city (Fig.3).

Key breeding sites of Aedes aegypti larvae differed from one area to another. In Haiphong city (100 km east of Hanoi), Aedes aegypti larvae were mainly found in concrete tanks (33.5%), in jars (29.7%) and in discarded objects (17.5%) (Un-published data). In Nghiadong commune, a rural area of Namdinh province (120 km south of Hanoi), Aedes aegypti larvae breeding sites were jars (58.4%), discarded objects (23.6%) and concrete tanks (14.87%). Surprisingly, larval breeding sites in Huongchu commune of Thua Thien Hue province (central coast of Vietnam) were ant traps (38.45%) and in Thanhbinh commune of Danang province these were toilet tanks (41.74%). In these areas, Aedes aegypti larval control measure should be different from those in Hanoi.
### Table 1. Surveillance results of Aedes aegypti and Aedes albopictus larval breeding sites in Hanoi, 1994 - 1997

<table>
<thead>
<tr>
<th>Containers</th>
<th>No of Types</th>
<th>No</th>
<th>%</th>
<th>Cont. (+)</th>
<th>% Avg No of Larvae</th>
<th>%</th>
<th>No of Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ae. aegypti</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ae. albopictus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Tanks</td>
<td>2475</td>
<td>38.93</td>
<td>211</td>
<td>26.15</td>
<td>8635</td>
<td>20.85*</td>
</tr>
<tr>
<td>2</td>
<td>Clay jars</td>
<td>1920</td>
<td>30.20</td>
<td>210</td>
<td>26.02</td>
<td>11684</td>
<td>28.22</td>
</tr>
<tr>
<td>3</td>
<td>Drums</td>
<td>1652</td>
<td>25.99</td>
<td>313</td>
<td>38.79</td>
<td>16707</td>
<td>40.35</td>
</tr>
<tr>
<td>4</td>
<td>Buckets</td>
<td>110</td>
<td>1.73</td>
<td>4</td>
<td>0.50</td>
<td>18</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>Discards</td>
<td>123</td>
<td>1.93</td>
<td>41</td>
<td>5.08</td>
<td>3708</td>
<td>8.96</td>
</tr>
<tr>
<td>6</td>
<td>Aquariums</td>
<td>77</td>
<td>1.21</td>
<td>28</td>
<td>3.47</td>
<td>654</td>
<td>1.58</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6357</td>
<td></td>
<td>807</td>
<td></td>
<td>41406</td>
<td>69</td>
</tr>
</tbody>
</table>

*underlined figures relate to key breeding sites.

### Figure 3. Key breeding sites of Aedes Aegypti, Hanoi, 1994-1997
Aedes aegypti larvae were found in all types of water containers. The number of larvae increased 2.2 times during the rainy season from April to November (Fig.4). These results are different from the theory propounded by Rakesh Katyal (1996) about “Primary and secondary” breeding sites of Aedes aegypti.

Conclusion
- Both Aedes aegypti and Aedes albopictus mosquito species were found in Hanoi, and Aedes aegypti was the dominant species (larvae accounted for 87.2% and adult mosquitoes accounted for 86.9%).
- Key breeding sites of Aedes aegypti in Hanoi were drums (40.35%), clay jars (28.22%), tanks (20.85%) and discarded objects (8.96%). Almost all Aedes albopictus larvae concentrated in clay jars (40.7%), discarded objects (24.25%) and drums (16.63%).
- There was no significant change in the types of Aedes aegypti breeding sites by month in Hanoi, but the number of larvae significantly increased during the rainy season from April to November.
- The relationship between Aedes aegypti indices and the number of cases was very low. Therefore, finding out other effective indices to predict DF/DHF outbreaks is necessary.

*This phenomenon is observed in regions of hot and dry climate. Aedes aegypti, being hygrophilic species, retreats to wet area (primary foci) during the hot season. In Vietnam, this phenomenon may not occur, being a tropical monsoon region. – Editor
References


Interspecific Association Between Aedes aegypti and Aedes notoscriptus in Northern Queensland†

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Abstract

During 1989-1990, surveys of artificial and natural water containers were undertaken to detect Aedes aegypti infestations in Townsville, Charters Towers, Mingela and Ravenswood localities. In doing this, the extent of interspecific association between Ae. aegypti and Ae. notoscriptus was measured on the basis of presence/absence data, and on their relative numbers. The distribution of these two species was significantly different (P<0.05 - <0.001), with containers having Ae. aegypti and Ae. notoscriptus breeding separately numbering more than the containers having co-breeding of the two species. This indicates a negative association. An analysis of the relative numbers of the two species by Sorensen’s coefficient gave values from 0.15 to -0.751, indicating little or no association. Statistical modelling of container records indicated that immature densities of Ae. aegypti in the presence or absence of Ae. notoscriptus or other species were not significantly different. In contrast to research suggesting that competitive displacement may occur in the temperate regions of New South Wales, little evidence could be found for this under the north Queensland conditions where temperatures are higher.

Key words: Aedes aegypti, Aedes notoscriptus, Interspecific association, Queensland, Australia

Introduction

Aedes aegypti is recognized as the major vector of dengue viruses[1] globally (WHO, 1986), whereas Kay and Aaskov[2] have shown that it is also a competent laboratory

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vector of Ross River virus. On the other hand, the vector status of Ae. notoscriptus has risen in importance recently with the successful laboratory transmission of Ross River, Barmah Forest and Rift Valley fever viruses\(^3,4\) and multiple isolates during the 1994 Ross River epidemic in Brisbane\(^5\). This species is also recognized as a major vector of dog heartworm, Dirofilaria immitis\(^6\).

Both Ae. aegypti and Ae. notoscriptus immatures have been recorded from a diverse range of artificial and natural habitats with the latter species perhaps with a greater propensity for utilizing natural sylvan biotopes\(^6,7,8\).

From laboratory studies, Russell\(^9\) demonstrated that in temperate areas, Ae. notoscriptus may have a competitive advantage over Ae. aegypti. During the course of field studies of Ae. aegypti in north Queensland between 1989-90, we also had the opportunity to examine the co-breeding of these and other species in a range of artificial containers in peridomestic habitats. By analysing the presence/absence records and by comparing the relative abundance of immatures, we were able to assess the evidence of competitive displacement in warmer tropical conditions.

Materials and Methods

Localities

Townsville (19° 15'S, 146° 50'E) is a coastal city of approximately 121,000 people and Charters Towers, a provincial town with 10,000 people lies 100 km south-west. The railway settlement of Mingela (pop. 80) and the former gold rush town of Ravenswood (pop. 180) are approximately 50 km and 90 km east and south-east, respectively, of Charters Towers.

Premises surveys

House-to-house surveys were carried out from February to March 1989 in seven suburbs of Townsville and in all four localities from February to August 1990. Details of frequencies of different container types and numbers of immatures collected are presented elsewhere\(^10\). Positive samples with up to 100 immatures in small-to-medium-sized containers up to 5 L capacity were sieved through a 100 µm mesh net, upturned into a white tray and counted directly. Samples of over 100 immatures were usually estimated on the basis of a 50% sub-sample.

For larger containers, e.g. 200 L drums, rainwater tanks, specialized nets were used. For drums, a 10 x 20 cm net of similar mesh size was swept once around the perimeter of the surface layer using the methods already described\(^11\). A 20 x 30 cm net was swept 3 times around the surface layer of rainwater tanks. Correction factors, estimated on the basis of mark-release-recapture studies of known numbers of Giemsa-stained larvae, were released into the larger containers and the proportions of the marked to unmarked larvae were used to establish correction factors for net samples\(^12\). From this, absolute numbers were estimated.

For identification, first instars were allowed to moult to seconds before examination, but second to fourth instars were identified directly at 40x magnification. Pupae were allowed to eclose to adults. Where both Ae. aegypti and Ae. notoscriptus were present in a container, the proportion of each was used to estimate the absolute numbers in the containers.
Measurement of interspecific association between Ae. aegypti and Ae. notoscriptus

The method of Fager\(^{(13)}\) detailed by Southwood\(^{(14)}\) was used to explain the independence or association of the two species. If there is an association, one would expect to see different proportions of the positives for Ae. notoscriptus depending on whether Ae. aegypti was present or not. If there is no association, one would expect the same proportions for Ae. notoscriptus irrespective of the fact whether Ae. aegypti was present or not. Two by two contingency tables were drawn up with the most abundant species, Ae. aegypti positive, occupying cells a and c and corrected \(\chi^2\) tests were used to determine statistically significant differences.

Cell d (neither Ae. aegypti nor Ae. notoscriptus present) was calculated on the basis of the positive containers only and not on the total number of wet negative containers. This was done to ensure uniformity of habitat under consideration as many wet biotopes, e.g. rainwater tanks, were screened or had recently been wet, e.g. tins, trays under plant pots, by rainfall or garden sprinkling. However, all wet containers, whether negative or containing other mosquito species, were recorded.

The proportion of individuals occurring together was determined using Sorensen's coefficient \(I\)\(^{(15)}\) as modified by Southwood\(^{(14)}\). Here -1 means no association and +1 means complete association. The following formula was used:

\[
I = 2 \left( \frac{J}{A+B} - 0.5 \right)
\]

where \(J\) = number of larvae of Ae. aegypti and Ae. notoscriptus in positive containers where both species are present, and

\[
A \text{ and } B = \text{ total larvae of both Ae. aegypti and Ae. notoscriptus in all positive containers.}
\]

Explanatory regression models were developed for the logarithms of numbers of Ae. aegypti immatures per positive container\(^{(16)}\). Poisson multiple regression was used with the following explanatory models in mind: (i) a series of simple Poisson regressions in which the variables were considered separately (unadjusted model), and (ii) a fully adjusted model in which all possible explanatory variables were considered simultaneously. The variable considered in this context was co-breeding, but in the adjusted model, region, suburb, water volume, organic detritus, pH, temperature, degree of shade and rain during (or just before) the survey were also considered.

Results

A total of 567 containers were found positive for mosquito immatures from the 1349 premises inspected (Table 1). Of these, 451 contained Ae. aegypti, alone (270) or cohabiting with Ae. notoscriptus (138), and/or with other species (43). Sixty-five containers had Ae. notoscriptus as the sole species present. Fifty-one containers had Cx. quinquefasciatus Say, An. annulipes s.l. Walker, Ae. quasirubithorax (Theobald) group or the predacious Tx. speciosus (Skuse) or Cx. halifaxii Theobald alone or as mixed populations.
The distribution of *Ae. notoscriptus* positive containers from all localities sampled in 1989-90 was significantly different ($\chi^2 = 24.95$, df = 1, $P < 0.001$) in the presence (138/451 = 30.6%) or absence (65/116 = 56.0%) of *Ae. aegypti*. When this analysis was limited to Townsville containers for 1989 ($\chi^2 = 4.98$, df=1, $P < 0.05$) and 1990 ($\chi^2 = 6.11$, df=1, $P < 0.025$), or for the rural towns ($\chi^2 = 16.57$, df=1, $P < 0.001$), the outcome was similar. This result therefore provided sufficient justification to further explore any interspecific associations, especially with respect to repulsion, as product of cells bc (both species breeding alone) was greater than ad (both species cohabiting and other species present).

The following numbers of wet containers with neither *Ae. aegypti* nor *Ae. notoscriptus* recorded for Townsville 1989 and 1990, and for Charters Towers, Mingela and Ravenswood were 529, 710 and 866 respectively. When these were substituted as for the values used in cell d, ad > bc all $\chi^2$ analyses were significant to $P < 0.0001$, indicating affinity.

When the relative abundances of *Ae. aegypti* and *Ae. notoscriptus* were considered using modified Sorensen's coefficients (Table 2), the values were as follows, indicating little association between the two species: combined (-0.392), Townsville 1989 (0.15), Townsville 1990 (-0.01) and Charters Towers, Mingela, Ravenswood (-0.751).

For the four major biotopes, plant pot bases, buckets, plastic ice cream containers and tyres (Table 3), simple Poisson regressions of logarithmic numbers of *Ae. aegypti* as the sole occupant or with *Ae. notoscriptus* and/or other species were non-significant ($P > 0.1$).

**Table 1.** Distribution of *Ae. aegypti* and *Ae. notoscriptus* from positive containers found in Townsville (1989 and 1990) and for combined data for Townsville, Charters Towers, Mingela and Ravenswood.

<table>
<thead>
<tr>
<th>Survey</th>
<th>Species</th>
<th>+</th>
<th>-</th>
<th>Total</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All localities</td>
<td><em>Ae. notoscriptus</em></td>
<td>138</td>
<td>65</td>
<td>203</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>313</td>
<td>51</td>
<td>364</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>451</td>
<td>116</td>
<td>567</td>
<td></td>
</tr>
<tr>
<td>Townsville 1989</td>
<td><em>Ae. notoscriptus</em></td>
<td>42</td>
<td>12</td>
<td>54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66</td>
<td>5</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>108</td>
<td>17</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Townsville 1990</td>
<td><em>Ae. notoscriptus</em></td>
<td>49</td>
<td>17</td>
<td>66</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>11</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>145</td>
<td>28</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Charters Towers, Mingela, Ravenswood</td>
<td><em>Ae. notoscriptus</em></td>
<td>47</td>
<td>36</td>
<td>83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>151</td>
<td>35</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>198</td>
<td>71</td>
<td>269</td>
<td></td>
</tr>
</tbody>
</table>

*Corrected $\chi^2$ test.
**Interspecific Association Between Aedes aegypti and Aedes notoscriptus in Northern Queensland**

**Table 2.** Sorensen's coefficients of interspecific association between Ae. aegypti and Ae. notoscriptus in four north Queensland localities

<table>
<thead>
<tr>
<th>Locality</th>
<th>J</th>
<th>A + B</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>13,801</td>
<td>45,376</td>
<td>-0.392</td>
</tr>
<tr>
<td>Townsville 1989</td>
<td>4,688</td>
<td>8,173</td>
<td>0.15</td>
</tr>
<tr>
<td>Townsville 1990</td>
<td>5,504</td>
<td>8,173</td>
<td>-0.01</td>
</tr>
<tr>
<td>Charters Towers, Mingela, Ravenswood</td>
<td>3,609</td>
<td>29,030</td>
<td>-0.751</td>
</tr>
</tbody>
</table>

**Table 3.** Mean immature density (± standard deviation) per litre in containers with Ae aegypti alone, or cohabiting with Ae notoscriptus and other species, Townsville 1989-9 and Charters Towers, Mingela, Ravenswood 1990.

<table>
<thead>
<tr>
<th>Container type</th>
<th>Alone</th>
<th>Temp(°C)</th>
<th>Cohabiting</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ae.aegypti</td>
<td></td>
<td>Ae. notoscriptus</td>
<td></td>
</tr>
<tr>
<td>Plant pot base</td>
<td>89 ± 94(39)</td>
<td>25.7 ± 4.3</td>
<td>107 ± 96(18)</td>
<td>27.6 ± 2.3</td>
</tr>
<tr>
<td>Bucket</td>
<td>21 ± 36(39)</td>
<td>21.7 ± 6.2</td>
<td>91 ± 36(17)</td>
<td>23 ± 4.8</td>
</tr>
<tr>
<td>Ice cream</td>
<td>38 ± 43(15)</td>
<td>22.2 ± 5.3</td>
<td>155 ± 80(13)</td>
<td>108 ± 4.8</td>
</tr>
<tr>
<td>Tyre</td>
<td>149 ± 52(34)</td>
<td>21.6 ± 5.5</td>
<td>116 ± 36(13)</td>
<td>76 ± 3.3</td>
</tr>
</tbody>
</table>

**Discussion**

Hurlbert\(^{17}\) pointed out that in order to measure the degree of association between two species, the analysis of presence-absence data is preferable to that of relative numbers, whereas Southwood\(^{14}\) indicated that both methods should be employed and examples discussed where values for cell d in the presence/absence analyses have been inflated through the inclusion of data for non-uniform habitats. We acknowledge that the inclusion of containers only found positive for mosquito breeding may result in an under-estimation of cell d (and consequently biasing the analysis towards significance and a negative association), we are assured that the containers under consideration are a homogeneous group. On the other hand, inclusion of all wet containers, some of which were definitely not suitable, will bias the analysis even more towards a positive association. This would lead us to concur with Southwood\(^{14}\). Our corrected χ\(^2\) analyses for all localities suggest that Ae. notoscriptus prefers to fill niches not occupied by Ae. aegypti. There could be numerous reasons for this - from competitive displacement of immature stages\(^{10}\) to different adult ovipositional stimuli\(^{18}\) or pheromonal repellents\(^{19}\).
In laboratory studies, Russell\(^9\) found that \textit{Ae. notoscriptus} had a marginal advantage over \textit{Ae. aegypti} at 22°C but this situation was reversed at water temperatures of 28°C. Overall, he found that the predominant species in mixed cultures was usually at an advantage, but the hypothesis of competitive displacement was not proved. In our studies (mainly in water temperatures from 22-25°C, but reaching extremes of 10-33°C), the modified Sorensen's coefficients indicated little or no association based on the relative abundance of the two species. The coefficients for Townsville and the rural towns of Charters Towers, Mingela and Ravenswood were different, influenced by the presence of large numbers of \textit{Ae. aegypti} in key containers such as rain water tanks in these latter towns. The mean immature densities of \textit{Ae. aegypti} were not depressed significantly in the presence of \textit{Ae. notoscriptus}. Furthermore, statistical modelling of the container records against a variety of factors, including water volume, water quality as indicated by organic detritus, pH, temperature, shade, locality, predation and co-breeding\(^20\), did not indicate the presence of other mosquito immatures to be significant. In both the unadjusted and adjusted models, only water volume and water quality remained significant.

In our north Queensland sites where cohabitation of \textit{Ae. aegypti} and \textit{Ae. notoscriptus} occurred, the sites were usually shaded and under vegetation which provided an ample supply of nutrients. Drowned insects in various states of decomposition were also evident. Thus, we would conclude that these two congeners occupy a niche often characterized by abundant food resources. Although one would surmise that they coexist in stable equilibrium within the same trophic level, it should be noted that nothing is known of the aquatic feeding patterns of \textit{Ae. notoscriptus}. Christophers\(^21\) has described three modes of feeding in \textit{Ae. aegypti}, i.e. filter feeding, gnawing and browsing. Nevertheless, since \textit{Ae. aegypti}-transmitted epidemics of dengue have been recorded for the region since 1879\(^22\) and \textit{Ae. notoscriptus} is an indigenous species, this in itself suggests that an equilibrium has been reached. Otherwise it would have been displaced.

**Acknowledgments**

We thank Dr S. Ritchie and Mr C. Jennings for the manuscript review and Mr S. Forsyth for computing assistance. This study forms part of Dr Tun-Lin’s Ph.D. thesis, which was funded jointly by WHO and the National Health and Medical Research Council, Canberra.

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Space Sprays for the Control of Aedes aegypti in South-East Asia and the Western Pacific

By

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Abstract

Space sprays for the control of adult mosquito populations are widely used by mosquito control groups throughout the world both for the pest and vector species. In the South-East Asia and Western Pacific regions, adulticiding is especially used to control vectors during epidemic outbreaks of mosquito-borne arboviruses such as dengue and Japanese encephalitis. Space sprays may be applied as thermal fogs in which kerosene or oil is used as a carrier for insecticides which produce dense fogs of droplets or as ultra low volume (ULV) sprays in which fine droplets of insecticide concentrate are applied. A review of the literature shows that carefully planned ULV applications have successfully controlled adult mosquito populations under the ecological conditions of eastern Asia. The indoor, sequential application of ULV concentrates have provided long-term control of Aedes aegypti populations in Thailand for a period of some months.

Space spray applications should be carefully planned, timed, supervised and evaluated by professional staff if they are to be effective. Equipment should be well-maintained. With the spread of Aedes stegomyia resistance to both the organo-phosphorus and pyrethroid insecticide groups, there is need for field trials of new compounds and new formulations.

Key words: Mosquito vectors, control, space sprays, evaluation, efficacy, insecticides, ULV, thermal fogs

Introduction

Virtually every urban vector control programme in the South-East Asia and Western Pacific regions makes extensive use of insecticidal space sprays. These are mostly intended to achieve rapid control of the adult populations of the principal mosquito vector of dengue, Ae. aegypti, especially during the epidemic outbreaks of dengue haemorrhagic fever. As there have been questions raised in the American Region with regard to the efficacy of this type of control, this paper will consider the methods and materials used in Asia and review the results obtained in field trials and operational conditions for the control of adult Stegomyia.
The principles of Aedes aegypti control

Aedes Stegomyia populations can be controlled either by disposing of the containers which are their larval habitats - 'source reduction' - by applying larvicides to the containers or by space sprays to control adult mosquitoes.

Despite the desirability of eliminating the places in which mosquitoes breed, it is unlikely that the number of larval habitats can be reduced to levels which will control mosquito populations under the ecological conditions of southern Asia. A large proportion of the Ae. aegypti breeding throughout the region takes place in containers used for storing water for household purposes; these cannot, of course, be readily or easily disposed of. Attempts to control such sources have not been successful under operational conditions of the region\(^1,2\). In areas lacking piped water supply to the houses or in which there is only an irregular provision of water, storage of water for household use remains a necessity. In addition, municipal services in the region are often unable to cope with the disposal of waste containers in which much mosquito breeding also occurs. The cooperation of the community in preventing mosquito breeding is sought but is difficult to obtain under existing conditions. Inhabitants throughout the region have also been urged to cover their water jars to prevent oviposition but it now appears that covered containers are likely to have more larvae in them than those without covers\(^3,4\).

The enormous number of containers in urban areas in which Ae. aegypti may develop, makes control by larviciding costly and difficult. Even if done very carefully, larviciding will have only a delayed effect on the densities of the adult vector populations and would thus not be a satisfactory means of controlling an epidemic outbreak of DHF. Furthermore, there is a declining acceptance by inhabitants of the application of chemical larvicides to household water containers.

Residual sprays, as used in mainly rural areas against Anopheles vectors of malaria, would be far too costly to apply in the vast numbers of rooms in buildings in urban areas. Most control operations have therefore little choice other than the use of adulticides as space sprays; most control organizations will continue to rely on them as no immediate alternative appears to be feasible or available. It is, therefore, important to make sure that adulticide applications are as efficient as possible.

Kilpatrick et al. (1970)\(^5\) has observed that epidemics of certain vector-borne diseases can be stopped or drastically curtailed if the infected vector population is reduced or eliminated. He and his colleagues carried out a series of field trials in Thailand which demonstrated rapid and effective control of adult mosquito populations by ultra low volume applications and these will be reviewed.

Methods of applying space sprays

Thermal fogs

Thermal fogs are produced by equipment in which an insecticide dissolved in an oil with a suitably high flash point is vapourized
when injected into the high-velocity stream of hot gases. Malathion has been the most commonly-used insecticide, usually applied by hand-carried Swingfog thermal generators, or, for larger areas, by vehicle-mounted generators. The hand-carried foggers usually have a pulse-jet engine (WHO 1990). Applications should be done early in the morning before thermal convection currents lift the fog from the ground level. Adult mosquito populations will generally recover rapidly unless the foggings are repeated; programme using thermal fogs should repeat applications every four days to maintain Ae. aegypti at low levels.

Thermal fogging is widely used by vector control organizations throughout the region. These thermal fogs are highly visible and inhabitants of the treated area perceive their application as an effort by the authorities to reduce mosquito populations. Unfortunately, thermal fogging are often applied late in the day (at a time when more people can see them) rather than early in the morning, they are not repeated frequently enough, and their efficacy is not evaluated to determine when retreatments are necessary. Nevertheless, if properly applied, thermal fogging can provide effective, if only short, periods of control. Applications of malathion 4% and fenitrothion 1% resulted in good reductions of natural infestations of mosquitoes in Bangkok; even better results were obtained with thermal fogs of pirimfos-methyl in Malaysia. Use is also being made of pyrethroids such as resigen, permethrin, cypermethrin and lambdacyhalothrin which can produce a rapid knock down of adult mosquitoes.

**Ultra low volume (ULV) fogs**

Also called “cold fogs” as no heat is used to produce them, ULV application equipment uses technical or high concentrations of insecticides which produce large numbers of droplet particles into the air, each of which, when of the right diameter, carries a dose lethal to the mosquito upon which it impinges. Mount et al. (1968) emphasized that ULV aerosols are advantageous as they have no need of oil solvents or carriers as with thermal fogging, the amount of spray solution or mixture that has to be applied is much smaller than thermal fogs, and ULV applications have no need of mixing. Early field trials carried out in the USA showed ULV applications to be at least as effective or more effective against both caged and free-flying Ae. taeniorhynchus.

In order to determine if ULV applications would be effective against Ae. aegypti in South-East Asia, trials were carried out in Thailand, first by aerial applications. The largest trial covered 18 km² of the city of Nakhon Sawan with some 9,000 houses, which were treated by two applications, four days apart, of 95% malathion as an ULV spray at a concentration of 438 ml/ha by a C47 aircraft. The Ae. aegypti landing counts before treatment were 8.6/man hour and premise indices were as high as 94%. The landing rate was reduced by 95% to 99% after the applications and remained low for ten days. The extent of the reduction of the mosquito populations was rapid and quite impressive; however, the aerial applications were expensive and depended on the availability of a large spray aircraft and a highly trained crew. Studies were therefore made on the efficacy of ULV ground application methods.
Pant et al. (1971)\(^{(11)}\) evaluated the efficacy of ground-applied ULV malathion aerosols in Bangkok using a Leco ULV generator. Unlike most previous ULV trials which had been carried out in open fields, these trials were carried out among the buildings in two small residential suburbs of Bangkok. Later the large suburb of Huay Kwang was treated several times and, finally, the whole city of Sri Racha was treated three times. It was found that excellent control of adult mosquitoes could be obtained at a dosage of 438 ml/ha of fenitrothion concentrate. Two ULV applications carried out three days apart enabled the adult *Ae. aegypti* population to be reduced by 99%, and it was three weeks before it regained its pre-treatment level.

These trials were followed by a study of the effect of ULV applications to the interior of houses\(^{(12)}\). A small hand-carried ULV machine was used to apply fenitrothion at 5.4 ml/house in the first application and, two weeks later, at 13.2 ml/house. After the second application, *Ae. aegypti* densities in the treated houses were very low for a period of two to three months.

Because of the persistent effect of this type of treatment, a further trial was carried out in Bangkok in an area of 1,300 houses and 10,000 inhabitants using a vehicle-mounted ULV LECO machine. Five sequential applications of fenitrothion ULV concentrate were made at intervals of 11 to 49 days and an effective control of *Ae. aegypti* populations was obtained for 4 days to 4 months\(^{(12)}\). The authors observed that sequential ULV applications could suppress vector populations throughout the rainy season and these had operational and cost advantages over larviciding.

Later, sequential applications were made in the Suitisan area of Bangkok, covering an area of 20 ha with 1,500 houses and 11,500 inhabitants. Two indoor ULV applications of fenitrothion were made at a rate of 0.1 ml/m\(^3\) of room space 14 days apart. A complete control of *Ae. aegypti* as measured by the landing rates and oviposition traps lasted for 6-7 months after the treatment; densities were substantially reduced up to a year, and recovery was slow even 16 months after the applications\(^{(13)}\).

The use of ULV applications in urban areas, especially the operational-scale field trials carried out in the South-East Asia and Western Pacific regions, was extensively reviewed by Gratz (1991)\(^{(14)}\), who emphasized their utility for the control of epidemic outbreaks of arboviruses.

For reasons which are difficult to perceive, little use has been made in Thailand of these ULV application procedures which were shown to be both effective and economical. By 1993, vector control budgets had been reduced despite the continuously rising trend of DHF cases in the country. Because of the increased trend, a reappraisal of the dengue vector control methods in use and the adoption of ULV adulticide control methods that have been shown to be effective in large-scale trials would seem to be timely\(^{(15)}\).

Field trials with ULV application of newer pyrethroid insecticides have also shown considerable promise against *Ae. aegypti* adults\(^{(16,17)}\).
It has been shown that adulticiding against Ae. aegypti in the South-East Asia and Western Pacific regions, if properly carried out, can be highly effective; ULV concentrates can achieve an immediate and persistent control, particularly if sequential applications are made. Further, large-scale operational trials and demonstrations of the methods and materials shown to be successful should be encouraged. Vector control programmes must also train their control personnel in the most efficient methods of the application of adulticides and their evaluation along with other methods of control.

Wherever possible, integrated control making use of all appropriate and feasible methods should be carried out against Ae. aegypti populations. Inhabitants of dengue endemic areas should be encouraged to dispose of undesired containers wherever possible; where this does not achieve the aim of reducing Ae. aegypti adult populations, well-directed, efficient adulticiding should be carried out.

References
Susceptibility of Aedes aegypti to Insecticides in South Vietnam

By
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Abstract
During 1998-1999, studies on the susceptibility of Ae. Aegypti to some insecticides were conducted in three places in Nam Bo and eight places in Central Highlands, south Vietnam. Ae. aegypti was found to be susceptible to malathion and resistant to DDT and three insecticides of the pyrethroid group, i.e. permethrin, lambda-cyhalothrin and deltamethrin, in many places.

Key words: Aedes aegypti, pyrethroids, insecticides, South Vietnam

Introduction
Insecticides continue to be the main tool for the control of Ae. aegypti, the vector of DHF. These insecticides belong to the organophosphate group (fenthion, malathion, fenithrothion and temephos) and the pyrethroid group (deltamethrin, resmethrin, permethrin and lambda-cyhalothrin)\(^1\,2,3\). However, Ae. aegypti became resistant to DDT in early 1960s as a consequence of large-scale spraying under malaria control and Ae. aegypti eradication programmes. Ae. aegypti has been cross-resistant to pyrethroid insecticides and to temephos in many countries, but not to malathion\(^4\).

Both malaria and DHF were endemic in many mountainous and coastal areas of Vietnam where house spray and bednet treatment were practised for years. DDT was widely used before 1990 and then ICON, permethrin and deltamethrin\(^5\) were introduced. The present study, therefore, was aimed at determining the current status of Aedes aegypti to these group of insecticides in Nam Bo and Central Highlands, south Vietnam.

Materials and methods

Study areas
Three sites in Kien Giang and Dong Nai provinces in Nam Bo and 8 sites in Gia Lai,
Kon Tum and Dac Lac provinces in the Central Highlands were selected for the study in 1998 and 1999, respectively.

The eggs laid by blood-fed mosquitoes from the fields were brought to the laboratory for hatching, and the adults were used as per WHO standard bioassay tests[6]. Female mosquitoes unfed for one or two days were exposed to the insecticide-treated paper obtained from WHO for one hour. The per cent mortality count was done 24 hours after exposure. Cotton pads soaked in 10% glucose solution were provided during the recovery period of 24 hours.

Results

In Nam Bo

Ae. aegypti was found susceptible to malathion and highly resistant to DDT in this area. It was resistant, highly tolerant and susceptible to permethrin at one each of the three sites. It was also highly tolerant to lambda-cyhalothrin at one and susceptible in two of the three places. Ae. aegypti was resistant in one and susceptible to deltamethrin in two of the three places (Table 1).

In Central Highlands

Ae. aegypti was susceptible to malathion and highly resistant to DDT in all places in this area (Table 2). It was found to be resistant and highly tolerant to permethrin, respectively, in 7 and 1 out of eight places. It was found to be resistant and highly tolerant to lambda-cyhalothrin in 6 and 2 of the 8 places, respectively. Ae. aegypti was resistant, highly tolerant and susceptible to deltamethrin in 5, 2 and 1 of 8 places, respectively.

Table 1. Results of susceptibility tests on Ae. aegypti to some insecticides in Nam Bo, 1998

<table>
<thead>
<tr>
<th>No.</th>
<th>Places</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Permethrin (0.25%)</td>
</tr>
<tr>
<td>1.</td>
<td>Nam Thai, An Bien distr., Kien Giang Pr.</td>
<td>60 (30/50)</td>
</tr>
<tr>
<td>2.</td>
<td>Buu Hoa, Bien Hoa City, Dong Nai prov.</td>
<td>100 (50/50)</td>
</tr>
<tr>
<td>3.</td>
<td>Phu Lap, Tan Phu distr., Dong Nai prov.</td>
<td>92 (46/50)</td>
</tr>
</tbody>
</table>

(Number of dead/number of tested mosquitoes)

Py = Pyrethroid; OC = Organochlorine; OP = Organophosphate
Table 2. Results of susceptibility tests on Ae. aegypti to some insecticides in Central Highlands, 1999

<table>
<thead>
<tr>
<th>No.</th>
<th>Places</th>
<th>Permethrin (0.25%)</th>
<th>Lambda-cyhalothrin (0.1%)</th>
<th>Deltamethrin (0.025%)</th>
<th>PY Control</th>
<th>Malthion (5%)</th>
<th>OP Control</th>
<th>DDT (4%)</th>
<th>OC Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quyet Thang, Kon Tum Capital, Kon Tum prov.</td>
<td>22 (11/50)</td>
<td>52 (26/50)</td>
<td>64 (32/50)</td>
<td>0 (0/25)</td>
<td>100 (50/50)</td>
<td>0 (0/25)</td>
<td>20 (10/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>2</td>
<td>Plei Can Ngoc Hoi distr., Kon Tum prov.</td>
<td>12 (6/50)</td>
<td>74 (37/50)</td>
<td>82 (41/50)</td>
<td>0 (0/25)</td>
<td>100 (50/50)</td>
<td>0 (0/25)</td>
<td>2 (1/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>3</td>
<td>Hoa Lu, Pleiku city Gia Lai prov.</td>
<td>86 (43/50)</td>
<td>88 (44/50)</td>
<td>98 (49/50)</td>
<td>0 (0/25)</td>
<td>98 (49/50)</td>
<td>0 (0/25)</td>
<td>8 (4/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>4</td>
<td>Chu Se capital Chu se distr., Gia Lai prov.</td>
<td>50 (25/50)</td>
<td>84 (42/50)</td>
<td>90 (45/50)</td>
<td>0 (0/25)</td>
<td>98 (49/50)</td>
<td>0 (0/25)</td>
<td>6 (3/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>5</td>
<td>Kon Dung, Mangfang distr., Gia Lai prov.</td>
<td>72 (36/50)</td>
<td>66 (33/50)</td>
<td>54 (27/50)</td>
<td>0 (0/25)</td>
<td>100 (50/50)</td>
<td>0 (0/25)</td>
<td>8 (4/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>6</td>
<td>Buon Trap, Krong Ana distr., Dac Lac prov.</td>
<td>20 (10/50)</td>
<td>42 (21/50)</td>
<td>22 (21/50)</td>
<td>0 (0/25)</td>
<td>98 (49/50)</td>
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<td>4 (2/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>7</td>
<td>Thang Loi, Buon Me Thuot, Dac Lac prov.</td>
<td>14 (7/50)</td>
<td>64 (32/50)</td>
<td>78 (39/50)</td>
<td>0 (0/25)</td>
<td>100 (50/50)</td>
<td>0 (0/25)</td>
<td>0 (0/50)</td>
<td>0 (0/25)</td>
</tr>
<tr>
<td>8</td>
<td>Ea Drang, Ea H'leo distr., Dac Lac prov.</td>
<td>14 (7/50)</td>
<td>14 (7/50)</td>
<td>6 (3/50)</td>
<td>0 (0/25)</td>
<td>100 (50/50)</td>
<td>0 (0/25)</td>
<td>2 (1/50)</td>
<td>4 (1/25)</td>
</tr>
</tbody>
</table>

(Number of dead/number of tested mosquitoes)

Py = Pyrethroid; OC = Organochlorine; OP = Organophosphate
Susceptibility of Aedes aegypti to Insecticides in South Vietnam

Discussion
Ae. aegypti was susceptible to malathion and resistant to DDT in Nam Bo. This species was also resistant to some insecticides of the pyrethroid group in many places, particularly in Central Highlands. These results were comparable with the observations of Reiter and Gubler (1997)\(^4\). This phenomenon was possibly due to the extended use of these insecticides in the malaria and DHF control programmes. It was recommended that the susceptibility tests should be conducted on all insecticides before use. In addition, the cross-resistance of Ae. aegypti should also be checked.

Acknowledgment
We would like to express our thanks to Dr Le Dinh Cong, Director, and Dr Tran Duc Hinh, Chief, Department of Entomology, National Institute of Malaria, Parasitology and Entomology, for their active interest in and support to the study.

References
Mesocyclops of Vietnam
Part I - Laboratory Evaluation as Biological Agent for Control of Aedes aegypti

By
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** Department of Preventive Medicine, Ministry of Health, Vietnam
# Queensland Institute of Medical Research, Brisbane, Australia,
## Medical Committee, Netherlands-Vietnam
† Kwansei Gakuin University, Hyogo, Japan;
†† Museum and Institute of Zoology, Warsaw, Poland
+ Smithsonian Institution, Washington DC, USA

Abstract
Nine species of Mesocyclops reportedly present in Vietnam have been widely distributed; especially they are naturally present in certain drinking water containers such as concrete tanks, wells and jars. Mesocyclops can survive and develop in normal conditions and are good predators of first instars of Aedes aegypti larvae, the major vector of dengue fever/dengue haemorrhagic fever in Vietnam. In laboratory, a single Mesocyclops can kill the first-instar of Aedes aegypti larvae (on average) up to 41 by M. pehpeiensis, 37 by M. aspericornis, 31 by M. woutersi, 22 by M. thermocyclopoides, 21 by M. affinis and 16 by M. ogunnus during 24 hours. The studies suggest that Mesocyclops hold high potential as a biological agent for the control of Aedes aegypti.

Key words: Mesocyclops, Dengue haemorrhagic fever, Aedes aegypti, biological control, Vietnam

Introduction
In Vietnam, dengue fever/dengue haemorrhagic fever (DF/DHF) has currently become an important health problem. During the recent five-year period 1994-1998, the number of reported cases and the incidence rate increased considerably. The number of cases reported were 44,944 (1994); 80,447 (1995); 74,569 (1996); 107,188 (1997), 235,000 (1998) while the incidence rate per 100,000 population was
Mesocyclops of Vietnam: Part-I, Laboratory Evaluation for Control of Aedes aegypti

65.8 (1994), 115.5 (1995), 105.0 (1996), 148.9 (1997) and 312.3 (1998). Aedes aegypti is the main vector of DF/DHF virus transmission in Vietnam. Recent studies indicate that insecticide spraying had a very limited effect, considering its cost and impact on the environment\(^1\). Mesocyclops, has drawn attention as a biological agent during the recent years for the control of Aedes aegypti larvae in some countries, e.g. Tahiti, French Polynesia, Honduras, Brazil, Mexico, Puerto Rico, Australia and Trinidad\(^1\). In Vietnam, the predatory capacity of Mesocyclops was first detected in 1989. The present study lists the species prevalent in Vietnam and their laboratory evaluation as biological agents for the control of Aedes aegypti.

Materials and methods

Research studies had been conducted during 1989 through 1998 in the Laboratory of Entomology, Institute of Hygiene and Epidemiology, Hanoi.

Nets with mesh size of 200 µ were used to collect Mesocyclops from 458 different water containers such as ponds, lakes, wells, concrete tanks, clay jars and ornamental aquaria. Species were identified in the Natural Museum, Washington D.C., USA, the Zoo Museum, Warsaw, Poland, and the Laboratory of Entomology, Institute of Hygiene and Epidemiology, Hanoi.

In the laboratory, the predatory capacity of Mesocyclops species was studied in 325 experiments, based on larval survival counts within 24 hours after the release of 50 first instars Aedes aegypti larvae and one Mesocyclops into a glass container of 500 ml capacity.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mesocyclops species</th>
<th>Natural Habitat</th>
<th>Artificial Habitat</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pond/lake</td>
<td>River</td>
<td>Tank</td>
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<tr>
<td>1.</td>
<td>M. affinis</td>
<td>6</td>
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<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>M. aspericornis</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>M. ogunnus</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4.</td>
<td>M. pehpeiensis</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>M. thermo-cyclopoides</td>
<td>20</td>
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<td>12</td>
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<tr>
<td>6.</td>
<td>M. woutersi</td>
<td>22</td>
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</tr>
<tr>
<td>7.</td>
<td>M. ferjemurami</td>
<td>1</td>
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</tr>
<tr>
<td>8.</td>
<td>M. yenae</td>
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</tr>
<tr>
<td>9.</td>
<td>M. dissimilis</td>
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<tr>
<td>Total</td>
<td></td>
<td>64</td>
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</table>

Table 1. Species of Mesocyclops and their distribution by water containers in Vietnam
### Table 2. Geographical distribution of Mesocyclops by province in Vietnam

<table>
<thead>
<tr>
<th>Province</th>
<th>M. woutersi</th>
<th>M. aspericomis</th>
<th>M. thermo-cyclopoides</th>
<th>M. pehpeiensis</th>
<th>M. affinis</th>
<th>M. ogunnus</th>
<th>M. yenae</th>
<th>M. ferjemurami</th>
<th>M. dissimilis</th>
<th>No of species</th>
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<td><strong>Northern</strong></td>
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<tr>
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<td>16</td>
<td>17</td>
<td>13</td>
<td>9</td>
<td>2</td>
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<td></td>
</tr>
</tbody>
</table>
Results
Mesocyclops species and their distribution
From 1989 up to 1998, a total of 458 copepod samples were collected from different water containers. Fifteen species comprising 5 genera were identified, which included 9 species of Mesocyclops. The results are presented in Table 1.

The geographical distribution of Mesocyclops is presented in Table 2. Data in Tables 1 and 2 show that 6 out of 9 species had been widely distributed in different localities and water containers, and were available in drinking water containers as well. Every inspected province had at least 1 species and a maximum of 6 species.

Laboratory studies
Predatory capacity of Mesocyclops to Aedes aegypti larvae
Among the detected nine species, six were confirmed to be predatory to Aedes larvae. In the laboratory, these six species were tested for their predatory capacity to Aedes aegypti larvae (Table 3). They were M. woutersi, M. pehpeiensis, M. aspericornis, M. thermocyclopoides, M. affinis and M. ogunnus. The results also showed that Mesocyclops had not only eaten but also killed the Aedes larvae. Different species had different eating and killing capacities. During the same period of time, a M. pehpeiensis ate less (11.7) but killed more (29.9) larvae than other species, resulting in the highest predatory capacity, followed by M. aspericornis and M. woutersi. Experiments showed that a population of primary 10 Mesocyclops, after one month, could kill at least 350 first instar Aedes aegypti larvae per day during 10 days (every day 350 larvae had been added, and no one could survive after 24 hours).

Table 3. Predatory capacity of Mesocyclops to Aedes aegypti larvae - First instar

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of experiments</th>
<th>No. of Ae. aegypti larvae used</th>
<th>Average No. of larvae eaten by Mesocyclops in 24 hours</th>
<th>Average No. of larvae killed in 24 hours</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. woutersi</td>
<td>75</td>
<td>3750</td>
<td>20.57</td>
<td>16.03</td>
<td>36.60</td>
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<td>M. pehpeiensis</td>
<td>40</td>
<td>2000</td>
<td>11.70</td>
<td>29.90</td>
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<tr>
<td>M. thermocyclopoides</td>
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<td>2500</td>
<td>9.58</td>
<td>12.48</td>
<td>22.06</td>
</tr>
<tr>
<td>M. affinis</td>
<td>50</td>
<td>2500</td>
<td>11.30</td>
<td>10.42</td>
<td>21.72</td>
</tr>
<tr>
<td>M. aspericornis</td>
<td>60</td>
<td>3000</td>
<td>23.75</td>
<td>13.43</td>
<td>37.18</td>
</tr>
<tr>
<td>M. ogunnus</td>
<td>50</td>
<td>2500</td>
<td>8.48</td>
<td>7.54</td>
<td>16.02</td>
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<tr>
<td>Control</td>
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<td>1000</td>
<td>0</td>
<td>1.40</td>
<td>1.40</td>
</tr>
</tbody>
</table>
Discussion and conclusion

From 1989 through 1998, there were nine species of Mesocyclops reported for the first time in Vietnam\textsuperscript{[3,4]}. This is a good piece of information for people working on copepods, especially using them for biological control. Previous authors\textsuperscript{[5,6]} had reported the presence of only one species of Mesocyclops (\textit{M. leuckarti} (Claus)). The work of Maria Holynska\textsuperscript{[7]} showed that 21 species of Mesocyclops had been detected in Asia; therefore, new species were expected in Vietnam. Mesocyclops were naturally available in the drinking water containers of people (83.0\% of the collected Mesocyclops samples). That is in contrast with other developing countries and is the most important factor to promote the use of Mesocyclops for dengue vector control in Vietnam. Results obtained in laboratory conditions showed that the predatory capacity of Mesocyclops for the control of \textit{Aedes aegypti} was very high. Among the six species tested, \textit{M. pehepensis} had the highest predatory capacity.

References

Laboratory and Field Evaluation of Bacillus thuringiensis H-14 (Bt.H-14) Granule Formulation Against Aedes aegypti in Delhi, India

By
M.A. Ansari and R.K. Razdan
Malaria Research Centre Indian Council of Medical Research, 20, Madhuban, Delhi-110 092 (India)

Abstract
Laboratory and field evaluations of Bt.H-14 granule formulation were carried out against Aedes aegypti, the vector of dengue and dengue haemorrhagic fever in Delhi, India. The results of laboratory evaluation revealed 100% mortality of Aedes aegypti larvae @ 0.5 gm/m² within 24 hours of exposure in enamel trays. The field evaluation revealed that biolarvicide @ 0.5 gm/m² provided an effective control of this species for more than four weeks in evaporation coolers and disused tyres.

Key words: Vector control, Aedes aegypti, Bt.H-14, Evaporation coolers, disused tyres

Introduction
Aedes aegypti is the vector of dengue, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The control of this species by space spraying of chemical insecticides has not been successful because of deeper and secure resting habits of the species. Sustainable and cost-effective control can only be achieved by eliminating the breeding places using eco-friendly technologies with the help of civic organizations and involvement of the community. Earlier surveys carried out in the Delhi National Capital Region revealed that the maximum amount of breeding of Aedes

* Bt.H-14 granule formulation was received through the courtesy of Hoechst AgEvo Ltd., Hoechst Centre, 54A, Andheri Kurla Road, Post Box No. 9473, Andheri (East), Mumbai-400 093 (India)
‡ Aedes aegypti larvae are known to feed at sides and bottom of the containers. The containers although are of various sizes/shapes, but the volume of water can be calculated. Bti formulation under this study is generally for surface feeders, however the author has evaluated it against Aedes aegypti larvae - Editor.
Laboratory and Field Evaluation of Bt.H-14 Granule Formulation Against Aedes aegypti in Delhi

Aedes aegypti takes place in evaporation coolers in domestic and disused tyres and other rain-filled receptacles in peri-domestic situations during the monsoon and post-monsoon periods\(^1,2\). Bt.H-14 formulations have been found to be effective against mosquitoes in general and against Aedes aegypti in particular\(^3,4\). The Bt.H-14 has been reported to be safe for humans when the biolarvicide is used in potable water in normal dosages\(^5\). However, normal formulations of Bt.H-14 tend to settle rapidly at the bottom of water bodies and may require frequent applications. Recently new slow-release formulations have been developed to prolong the larvicidal activity, particularly for those breeding places which are not easily and frequently accessible. In view of this, studies were initiated to evaluate Bt.H-14 granule formulation under laboratory and field conditions against Aedes aegypti. Results of this study are presented in this paper.

**Materials and methods**

Laboratory-reared larvae of Aedes aegypti\(^6\) were used for bioassay tests. Twenty-five late II\textsuperscript{nd} and III\textsuperscript{rd} instar larvae were introduced in enamel trays (15x20 cm) containing about 500 ml water and 100 mg of larval food. Bt.H-14 @ 250, 500 and 1000 mg/m\(^2\) was applied on the water surface. The experiment was repeated 10 times when each dosage and larval mortality was recorded at 24, 48 and 72 hours post-exposed period. Corrected mortality was calculated by using the Abbott formula.

% reduction = 100 - ( C\textsubscript{1}/T\textsubscript{1} \times T\textsubscript{2}/C\textsubscript{2} )

where C\textsubscript{1} and T\textsubscript{1} are the pre-treatment density and T\textsubscript{2} and C\textsubscript{2} are the post-treatment density of III+IV instar larvae per dip in the control and treated habitats, respectively.

**Results**

**Laboratory evaluation**

The results of laboratory evaluation revealed that the formulation had shown a high larvicidal activity against immature mosquitoes. Of the three species tested, Aedes aegypti was the most susceptible followed by Cx. quinquefasciatus and An. stephensi. Bt.H-14 granule formulation @ 0.25 gm/m\(^2\) produced 100% mortality in evaporation coolers, and Mayapuri (Motia Khan) circle no.8, West zone, was selected for discarded tyres. Selection of the localities was decided on the basis of the breeding potential and operational convenience. The trials were conducted during August-October 1997 in 20 experimental and 4 control coolers and 36 experimental and 6 control tyres. The mean larval density was calculated on the basis of 5 dips/coolers and 6 pipette dips per tyre. Prior to the experiment the surface areas of coolers and tyres were measured along with the pre-spray density of larvae. The biolarvicide was applied @ 250, 500 and 1000 mg/m\(^2\) in both coolers and unused tyres and the post-spray density of I-II and III-IV instar larvae was recorded after 24 hours. Successive observations were made at an interval of one week. The percentage reduction was calculated by the following formula earlier described by Mulla\(^7\):

The Mehrauli rural circle No. 141, South zone, was selected for field trials of the biolarvicide in evaporation coolers, and Mayapuri (Motia Khan) circle no.8, West zone, was selected for discarded tyres.
Laboratory and Field Evaluation of Bt.H-14 Granule Formulation Against Aedes aegypti in Delhi

among all the three species of mosquitoes within 24 hours of post exposure. A lower dosage, i.e. 0.125 gm/m², also produced a 100% mortality in Aedes aegypti within 24 hours as against 94.0% and 98.0% mortality after 72 hours of post-exposure period for An. stephensi and Cx. quinquefasciatus respectively (Table 1).

Table 1. Bio-efficacy of Bt.H-14 granule formulation against larvae of Aedes aegypti under field conditions

<table>
<thead>
<tr>
<th>Dose in Mg/m²</th>
<th>% reduction after 24 hrs</th>
<th>I week</th>
<th>II week</th>
<th>III week</th>
<th>IV week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>98.5</td>
</tr>
<tr>
<td>500</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>97.0</td>
</tr>
<tr>
<td>250</td>
<td>100.0</td>
<td>100.0</td>
<td>92.0</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Note: Five replicates of each dose were made in enamel trays consisting of 200 IInd and IIIrd instar larvae.

Table 2. Bio-efficacy of vectobac granules formulation against Aedes aegypti in evaporation coolers under field conditions

<table>
<thead>
<tr>
<th>Day/ Week</th>
<th>I+ II (Instar)</th>
<th>III+ IV (Instar)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>@ 1.0 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>2.8</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>I day</td>
<td>0.08</td>
<td>1.5</td>
<td>0.07</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>III week</td>
<td>0.0</td>
<td>3.2</td>
<td>0.0</td>
</tr>
<tr>
<td>IV week</td>
<td>0.0</td>
<td>2.1</td>
<td>0.0</td>
</tr>
<tr>
<td>@ 0.5 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>1.3</td>
<td>2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Post-I day</td>
<td>0.1</td>
<td>1.5</td>
<td>0.13</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>III week</td>
<td>0.0</td>
<td>3.2</td>
<td>0.0</td>
</tr>
<tr>
<td>IV week</td>
<td>0.0</td>
<td>2.1</td>
<td>0.0</td>
</tr>
<tr>
<td>@ 0.25 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>1.5</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Post-I day</td>
<td>0.2</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>3.3</td>
<td>0.6</td>
</tr>
<tr>
<td>III week</td>
<td>0.5</td>
<td>3.2</td>
<td>0.9</td>
</tr>
<tr>
<td>IV week</td>
<td>0.1</td>
<td>2.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Note: 1. Percent reduction was calculated on the basis of IIIrd and IVth instar density.
2. E = Experimental coolers, C = Control coolers.
### Table 3. Bio-efficacy of Bt.H-14 granule formulation against Aedes aegypti larvae in disused tyres under field conditions

<table>
<thead>
<tr>
<th>Day/Week</th>
<th>I+II (Instar)</th>
<th>III+IV (Instar)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>@ 1.0 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>11.8</td>
<td>10.6</td>
<td>8.6</td>
</tr>
<tr>
<td>I day</td>
<td>0.3</td>
<td>8.6</td>
<td>0.3</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>8.9</td>
<td>0.0</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>11.2</td>
<td>0.0</td>
</tr>
<tr>
<td>III week</td>
<td>0.0</td>
<td>10.8</td>
<td>0.0</td>
</tr>
<tr>
<td>IV week</td>
<td>0.0</td>
<td>8.2</td>
<td>0.0</td>
</tr>
<tr>
<td>@ 0.5 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>8.6</td>
<td>10.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Post-I day</td>
<td>0.3</td>
<td>8.6</td>
<td>0.4</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>8.9</td>
<td>0.0</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>11.2</td>
<td>0.0</td>
</tr>
<tr>
<td>III week</td>
<td>0.0</td>
<td>10.8</td>
<td>0.0</td>
</tr>
<tr>
<td>IV week</td>
<td>0.0</td>
<td>8.2</td>
<td>0.0</td>
</tr>
<tr>
<td>@ 0.25 gm/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-0 day</td>
<td>6.6</td>
<td>10.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Post-I day</td>
<td>0.6</td>
<td>8.6</td>
<td>0.8</td>
</tr>
<tr>
<td>I week</td>
<td>0.0</td>
<td>8.9</td>
<td>1.2</td>
</tr>
<tr>
<td>II week</td>
<td>0.0</td>
<td>11.2</td>
<td>1.8</td>
</tr>
<tr>
<td>III week</td>
<td>0.4</td>
<td>10.8</td>
<td>2.5</td>
</tr>
<tr>
<td>IV week</td>
<td>0.7</td>
<td>8.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Note: 1. Percent reduction was calculated on the basis of IIIrd and IVth instar density.  
2. E = Experimental tyres, C = Control tyres.

### Field evaluation

The application of Bt.H-14 in evaporation coolers resulted in a high degree of reduction in density of the immature of Aedes aegypti; however, the persistence of biolarvicide was directly proportional to its dosage. Higher dosages, i.e. 1 and 0.5 gm/m², produced 100% reduction in the larval density of these species for about four weeks as compared to the untreated control. Lower concentration, i.e. 0.125 gm/m², also...
showed a high larvicidal activity against this species, but the percentage reduction was not as pronounced as with higher dosages. The percentage reduction @ 0.25 gm/m² was 71.8, 65.6, 56.8 and 46.8, respectively, during 1st-4th week as against 100 with 0.5 gm/m² (Table 2). The application of Bt.H-14 formulation in disused tyres @ 1, 0.5 and 0.125 gm/m² produced variable degrees of percentage reduction (83-100%) in the larval density of Aedes aegypti (Table 3). As observed earlier, the formulation @ 1 and 0.5 gm/m² also resulted in 100% reduction in the larval density up to four weeks in tyres and the reduction was consistent in successive weeks of post-exposure periods. The formulation @ 0.25 gm/m² also showed considerable larvicidal activity (47.7%-83.4%) in this habitat but a hundred per cent reduction in immature density was not evident.

**Discussion**

Laboratory and field trials clearly indicate that the Bt.H-14 granule formulation has broad spectrum activities against larvae of mosquitoes in general and against Aedes aegypti, the principal vector of dengue and dengue haemorrhagic fever in India, in particular. B. sphaericus, which has recycling properties, has not been found effective against the larvae of Aedes aegypti in comparison to Culex quinquefasciatus and An. stephensi(1,8,9). However, this formulation was most effective against Aedes aegypti @ 0.5gm/m² and the larvicidal activity persisted up to four weeks. Therefore, the formulation can be effectively used for the composite control of dengue, DHF, malaria and filaria vector species of mosquitoes in urban areas. As the formulation produced larvicidal activity for longer duration in domestic and peri-domestic habitats, it will be operationally feasible to treat specific breeding places at the onset of the monsoon, particularly in abandoned coolers and tyres, to prevent the rapid build-up of vector density and thereby of dengue and DHF epidemic. Nevertheless, before incorporating this method into the strategy for urban malaria control, pilot studies are required to be undertaken with conventional larvicides and oil to evaluate the relative efficacy, operational feasibility and cost-effectiveness of each method of intervention.

**Acknowledgements**

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**References**

Establishment of an Environmental Master Team to Control Dengue Haemorrhagic Fever by Local Wisdom in Thailand

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Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract
Dengue haemorrhagic fever (DHF) is endemic in Thailand with variable evidence in different provinces. Although case fatality rates have been brought down considerably in recent years, the sustainability of vector control programme has not been achieved. In 1998, an “Environmental Master Team” (EMT) for control of DHF based upon the wisdom of local communities was constituted at Ban Now Village, Maung district of Chaiyapum Province. The essential objectives of EMT was to establish linkages at the local level through the district administration and it comprised of 30 members drawn from different blocks. The EMT selected its own team leaders and advisers and worked on a voluntary basis. The health personnel imparted knowledge about DHF and its control through lectures/discussions and field demonstrations. The EMT arranged monthly meetings to resolve problems and was frequently assisted by experts from the health department. Following one year of implementation, the entomological indices showed a significant reduction. However the landing rates showed great fluctuations. This was due to lack of identification of “key containers” and their elimination. The study village did not report any DHF case during the implementation period. The experiment has been rated a great success in ensuring the sustainability of the DHF Control Programme.

Key words: DF/DHF, Environmental Master Team, Local wisdom, sustainability

Introduction
In Thailand, for almost 50 years after it was first recognized as a disease in 1949, DHF has spread across the country. It is considered an endemic disease in certain

* This study received support from World Health Organization, South-East Asia Regional Office.
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areas. With the availability of better medical and health care facilities to communities, there has been a sharp decrease in deaths and case fatality rates during the past few decades; however, the morbidity rate seems to have remained rather stable\(^1\). In 1998, the Chaiyapum province recorded 2292 DHF cases with a morbidity rate of 211.4 per 100,000 population. There were five children who died from the disease. In this province, the disease mostly affected children aged between 5-9 years, 10-14 years, and 0-4 years, respectively\(^2\). From experience, it was found that the DHF epidemics in Thailand peaked between November and April. In 1998, the Ministry of Public Health observed that the peak incidence increased sharply, particularly from June to August\(^3\), the same as in Chaiyapum province. However, these perceptions did not percolate down to the local community level and public health workers' level. Therefore, sustainable control failed to achieve its goals due to lack of good linkage between these two sets of workers\(^4\). Even though DHF control activities were launched and implemented many years ago, sustainability is still limited. In order to overcome this problem we developed the concept of the Health Belief Model\(^5\) (HBM) to convince villagers of the efficacy of DHF intervention and to undertake minimal levels of health promotion. The success of HBM requires the establishment of a core group of people to be responsible as an “Environmental Master Team” who conserve and save their community environment. The objective of the study was to establish an environmental master team which can help the community to reduce DHF morbidity. This model was tested in Ban Non in Maung district of Chaiyapum Province from December 1998 to January 2000.

**Study area**

Ban Non is located in Tambon Non Samran, Muang district of Chaiyapum province and has 392 households and a population of 1163. It is approximately 350 kilometers northeast of Bangkok. The village is fully equipped with facilities such as a health centre, Sub-district Administrative Organization, school, monastery, and a strong group of village leaders.

**Knowledge of DHF and its control among community**

As per the KAP study undertaken at the beginning of the project, villagers had a high level of knowledge about DHF (96.1%) (Tables 1 and 2). They knew about the fact that the Aedes mosquito transmits the disease (92.6%), about the symptoms (86.7%), and the severity of illness (93.1%). They also had perceptions of the risk of disease susceptibility (49.7%), and that the disease was life-threatening (36.0%). However, the incidence of mosquito protection behaviour among children was very low (20.7%).

**Vector breeding potential**

Water jars had no covers (52.9%), wastewater lacked drainage (78.3%) and the garbage was disposed by burning and discarding in the yard near the home. Mosquito indices, at the beginning, showed a House Index of 68%, a Container Index of 10.0% and a Breteau Index of 136 containers per 100 houses; the mosquito landing rate was 12.8.
**Table 1.** Characteristics of Ban Non study population, 1999 (N = 203 heads of household)

<table>
<thead>
<tr>
<th>Population characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age (year)</td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>20 (9.9)</td>
</tr>
<tr>
<td>30-49</td>
<td>87 (42.8)</td>
</tr>
<tr>
<td>50 and over</td>
<td>96 (47.3)</td>
</tr>
<tr>
<td>2. Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>74 (36.5)</td>
</tr>
<tr>
<td>Female</td>
<td>129 (63.5)</td>
</tr>
<tr>
<td>3. Education attained</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>178 (87.7)</td>
</tr>
<tr>
<td>Other</td>
<td>25 (12.3)</td>
</tr>
<tr>
<td>4. Occupation</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>186 (97.6)</td>
</tr>
<tr>
<td>Other</td>
<td>17 (2.4)</td>
</tr>
<tr>
<td>5. Annual Income (Baht)</td>
<td></td>
</tr>
<tr>
<td>&lt; 10000</td>
<td>26 (12.8)</td>
</tr>
<tr>
<td>10000 – 999999</td>
<td>150 (73.9)</td>
</tr>
<tr>
<td>100000 and above</td>
<td>27 (13.3)</td>
</tr>
</tbody>
</table>

**Socio-cultural cum economic factors**

The majority of houses in Ban Non village store domestic and drinking water in jars or containers without covers. We found that many houses had a number of rainwater harvesting containers. The reason was that they could not afford the 4000 - 6000 Baht fee for piped water supply (Government Sector). Environmental conditions were generally poor, there were no garbage disposal places, there was no wastewater drain and no village clean-up campaign.

**Materials and methods**

The first activity carried out in Chaiyapum was to identify the official channels for approaching the Provincial Health Officers who could establish linkages with all levels schools, village leaders, women’s groups and the President of the Sub-district Administrative Organization (SAO). They understood the objectives and perceived the need, and then initiated the selection process of the environmental master team.

In order to achieve our objectives, we started with a formative demonstration to identify what was important to the village and needed to be implemented at the village level. The study attempted to address DHF problems within the village committee, in order to take into account local perceptions of appropriate prevention techniques. We discussed with them and tried to make them understand the issues of environmental health; baseline data of the village concerning mosquitoes and disease, and the future health status, as affected by DHF. Utilizing their own social cohesion and wisdom we found appropriate ways to disseminate self-help decisions and processes for changed behaviour for...
sustainable disease control. Subsequently, a group of people was selected by villagers themselves to form an “environmental master team”. Commitment to teamwork was ensured by insisting that the group must comprise of people who were willing to work voluntarily, without added incentive. The team consisted of 30 members who each represented one of the 15 house-blocks in the village. Two members came from each house-block. The team selected advisers, comprising the village headman, a teacher from the school, the head of the health centre and the president of the SAO. The environmental master team was trained in all activities to be performed for one year. There were four main training sessions and monthly meetings during the year of study. The environmental master team periodically requested for supplementary training materials, and resource persons for advice and training.

For evaluation of the project, the measurement of vector density was based upon simple methods of mosquito larval and adult surveys by larval sampling procedures and landing counts per man-hour. The mosquito surveys were done four times throughout the study period.

Functioning of the environmental master team

After each training session, the environmental master team members could go back to their house-block to work with their people. The functions of an environmental team member were to:

- Introduce health education to villagers concerning DHF and preventive methods;
- Manage environmental conditions across the house-block;
- Create community awareness about Aedes larvae in water containers;
- Supply sand abate to villagers and explain application methods, and
- Link with team advisers, especially health personnel.

The team members would come to the monthly meeting to report their performance and problems faced by the house-block. During meetings, the team members could solve problems, for example, searching for a public space for dumping garbage (now in use), constructing a receptacle bin for public garbage (in the plan), raising funds for buying a garbage truck (in the plan) and raising a clean-up campaign in the village (now in place).

Members of the environmental team felt responsible for the health of other families. Meetings were organized by themselves every month, mostly at night time after their housework. Four training sessions were conducted mostly in the meeting room of the SAO. A monthly meeting was organized by school teachers, health officers and the President of the SAO.

Social cohesion in the village was a strong tie. Information education and communication (IEC) messages and activities were undertaken to advocate the need for community cooperation, and complete coverage and supervision by almost all families for the benefit of all children. At the end of the year, the winners of the beautiful-village contest were declared.
Table 3. Breteau index (BI) and House index (HI) of Ae.aegypti in Ban Non Village (experiment) compared to a nearby village (control), Dec.1998 - Jan. 2000.

<table>
<thead>
<tr>
<th>Village</th>
<th>Dec 98</th>
<th>May 99</th>
<th>Oct 99</th>
<th>Jan 00</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BI</td>
<td>HI</td>
<td>BI</td>
<td>HI</td>
</tr>
<tr>
<td>Ban Non</td>
<td>136.0</td>
<td>341.1</td>
<td>53.5</td>
<td>131.5</td>
</tr>
<tr>
<td>Control</td>
<td>114.3</td>
<td>297.9</td>
<td>100.0</td>
<td>262.1</td>
</tr>
</tbody>
</table>

BI = Number of positive containers per 100 households
HI = Number of positive containers per 100 population

Results

The results of the entomological survey are indicated under Table 3. Over the period: December 1998 to January 2000, the House Index, the Container Index and the Breteau Index all showed a reduction. But the mosquito landing rates remained unchanged, and sometimes showed fluctuations (Table 4). This was possibly due to lack of identification of “key containers” responsible for bulk production of vector species, and where elimination was not possible because of perpetual use. Interestingly, Ban Non village has shown no evidence of DHF cases since the implementation of the project.

Table 4. Landing rate (LR) of Ae.aegypti in Ban Non Village (experiment) compared to a nearby village (control), Dec.1998 - Jan.2000.

<table>
<thead>
<tr>
<th>Village</th>
<th>Dec 98</th>
<th>May 99</th>
<th>Oct 99</th>
<th>Jan 00</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR</td>
<td>LR</td>
<td>LR</td>
<td>LR</td>
</tr>
<tr>
<td>Ban Non</td>
<td>12.8</td>
<td>17.2</td>
<td>21.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Control</td>
<td>8.7</td>
<td>10.5</td>
<td>20.2</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Landing rate = Number of mosquitoes/man/hour

Conclusions

Villagers, village leaders, health personnel and school teachers who contributed much of their time for project activities were deeply involved and motivated by this project from the beginning. This project was implemented for one year because of a very limited budget. The best that could be done was to establish an environmental master team, train the team, carry out an entomological survey, conduct a sociological baseline survey and evaluate the results. For ensuring a sustainable programme, the villagers need more time to concentrate on their activities on environmental management and reduction of mosquito breeding sources. The most important outcome of the project is that they would like to prevent the occurrence of DHF cases in their village. However, a lot unfinished work still remains to be completed for achieving the objective of the project. Hence, it is recommended that:

1. The constraints encountered during the implementation namely: time limitations; starting with a simple method, and focusing on the objective be minimized and/or removed;
(2) Health education materials, manuals and guidelines related to DHF kept in the health office should be distributed to people in communities;

(3) The government should facilitate the rural people having access to public water supply at very cheap rates in their houses. This will result in reduction of the number of water containers in households, and

(4) The researcher must conduct an evaluation of the environmental master team, and test whether it can perform sustainable activities.

It is recommended that this project continued over a long term in order to achieve environmental and human behavioural changes on and thus should be carried out on a continuous basis. Even though Thailand has limited resources, involvement should be a high priority in endemic areas and in areas at high risk for DHF.

References
Dengue Shock Syndrome: Clinical Manifestations, Management and Outcome - A Hospital-Based Study in Jakarta, Indonesia

By
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Department of Child Health, Faculty of Medicine, University of Indonesia
Dr Cipto Mangunkusumo Hospital, Jakarta

Dengue shock syndrome (DSS) is one of the serious complications in cases of dengue haemorrhagic fever (DHF). Shock is the primary evidence in DHF, while other organs' involvement is secondary to shock. Depending upon the host's resistance and possibly the viral virulence, shock syndrome, if not managed, can prove terminal (irreversible). Early detection and prompt treatment can give a good prognosis. The objectives of this study are to show the clinical manifestations, case management and the outcome of DSS cases.

An observational, cross-sectional study was conducted on DSS cases hospitalized at the Paediatric Intensive Care Unit, Child Health Department, Faculty of Medicine, University of Indonesia, and Dr Cipto Mangunkusumo Hospital, Jakarta, during January-June 1998. The diagnosis of DSS was established by the WHO diagnosis criteria (1997) and confirmed by the haemagglutination inhibition serological test. Virus detection was done during the period of study by PCR examination. There were 188 DSS cases included in this study of which 46.1% were male and 53.9% female; 85% had good nutrition. The age group distribution was: 10 cases were below 1 year of age, 1-4 years old accounted for 29% while those above 10 years accounted for 15.40%. The highest proportion of the cases (40%) belonged to the 5-9 years age group. Those below 1 year of age had the highest mortality as compared to other age groups, while no one above 10 years of age died. Twenty per cent, 66% and 11% cases had primary, secondary and presumptive dengue infection, respectively. DEN-3 was the predominant serotype.

The deceased suffered from unconsciousness, tachycardia, tachyrea, decreased capillary refill, decreased body temperature, hypertension, and oliguna,
which were the major symptoms. Among the 188 DSS cases, 63.2% recovered from shock within less than 80 minutes. Thirty-nine cases suffered from irreversible shock, of whom 25 died. The overall DSS case mortality was 19.7%. Gastrointestinal bleeding, encephalopathy and respiratory failure were the most common causes of death, which was due to delayed treatment and hospital admission as referral cases without adequate treatment.

Reference
DHF remains a major public health problem in Indonesia, especially in Surabaya. A steep increase in the incidence of DHF was observed in Surabaya, from 753 cases (IR. 39.6/100,000 population) in 1973 to 3667 cases (IR. 122/100,000 population) in 1998. This rise was facilitated by increased migration and population growth and the widespread prevalence of the vector, *Aedes aegypti*. Eighty per cent of the villages in Surabaya are DHF endemic areas where larval indices exceed 85%.

The objectives of this study were to analyse the correlation of the housewives’ awareness about DHF and its control. This was assessed through a knowledge, attitude and practice (KAP) study. The survey was an analytical, observational, cross-sectional study. The study population comprised of 100 individuals selected through sampling. The data was analysed using multiple logistic regression with stepwise method. Dependent variables were the housewives’ knowledge, attitude and practice in controlling DHF. Independent variables were the age and education of housewives, household income, and the presence of cases in the household or their neighbours.

The results were as follows:

(1) A significant correlation was found between the household income and the presence of DHF cases with the housewives’ knowledge about controlling DHF: (a) every increase of Rp.100,000 in the household income resulted in better knowledge (as much as 1.34 times), and (b) housewives with the presence of cases had 0.28 times less knowledge than housewives without cases.

(2) A significant correlation was found between the presence of cases with the housewives’ attitude towards controlling DHF. Housewives with the presence of cases had 0.5 times lower attitude than housewives without cases.

(3) A significant correlation was found between the housewives’ practice in controlling DHF. Every increase
of 10 years in the age of a housewife resulted in a better practice by 2.88 times.

(4) A significant correlation was found between the presence of cases with the housewives' behaviour in controlling DHF. Housewives with the presence of cases had a behaviour level 0.41 times lower than the housewives without cases.

(5) A significant correlation was found between the housewives' knowledge with their practice in controlling DHF. Better knowledge had better practice as high as 3.43 times.

It was concluded that while DHF had been endemic in Surabaya for almost 30 years, the control and prevention of the disease had not been fully understood by all people. It is important, therefore, that for the success of the programme of elimination of mosquito breeding places, people's knowledge of DHF and its control should be enhanced by campaigns using available mass media as well as community organizations.
Status Report on DF/DHF During 1998 in the National Capital Territory of Delhi, India

By
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#National Institute of Communicable Diseases, 22 Shamnath Marg, Delhi-110 54

The National Capital Territory (NCT) of Delhi, the Capital of the Republic of India, is situated on the banks of the river Yamuna. It covers an area of 1485 sq km, of which 900 sq km is urban and the rest is classified under the rural category. The city has experienced a phenomenal growth in its population, and as a centre offering vast economic opportunities has been attracting migrants not only from its immediate neighbourhood but also from far off places. The population of Delhi is now estimated to be much above 10 million.

Delhi has been endemic for dengue, and the first DHF outbreak was reported in 1988 with 33% mortality among children admitted into hospitals[1]. The last outbreak of DHF occurred in the NCT of Delhi in 1996 when more than 10,000 cases and 450 deaths were recorded[2].

Comprehensive studies on the prevalence of Aedes aegypti, the vector responsible for DF/DHF, carried out in Delhi revealed a high breeding potential of the species. While the species is present all the year round, but the seasonal peak coincides with the rainy season from July to October[3,4].

After the 1996 outbreak, dengue was declared a dangerous disease under the Delhi Municipal Act, which enjoins upon all medical practitioners/hospitals and other persons to provide information to the Municipal Health Officer and the National Anti-Malaria Programme (NAMP) which is the nodal agency for the monitoring of dengue at the national level.

The present communication contains the disease incidence as reported by all the hospitals of the city and the entomological indices collected by NAMP.

Dengue incidence
The blood samples of clinical dengue cases were confirmed in different hospitals by
using the Pan-Bio, Australia, test kit for IgM antibodies. The dengue cases were also diagnosed at the National Institute of Communicable Diseases by using antigens from the National Institute of Virology, Pune.

During 1998, a total of 518 cases were reported, out of which 332 cases (64%) were found to be positive for dengue. The monthly incidence of dengue cases and deaths are shown in Fig.1. The age-wise and sex-wise incidence of dengue cases is given in Fig.2. It is apparent that the occurrence of the cases started with the onset of the rainy season (July) and peaked in November. Nearly 69.5% of the cases were in the age-group of 14 and above. In all age-groups males outnumbered the females.

Longitudinal vector surveillance studies undertaken by the NAMP in all divisions of the city, namely, Delhi Municipal Corporation area, New Delhi Municipal Committee area and the Defence area, showed heavy breeding of Aedes aegypti in all the areas. The monthly cumulative container indices fully synchronized with the disease incidence (Fig.3).

**Figure 1.** Monthwise DF/DHF cases during 1998 in NCT Delhi
**Figure 2.** Age and sexwise distribution of DF/DHF cases in NCT Delhi during 1998

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1-4</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>4-9</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>9-14</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>&gt;14</td>
<td>162</td>
<td>69</td>
</tr>
</tbody>
</table>

**Figure 3.** Seasonal fluctuations of breeding infestation of Aedes Aegypti during 1998 in NCT Delhi
Therefore, it may be concluded that for effective control of DF/DHF in NCT, Delhi, there is a need for building-up a comprehensive vector control programme based on source reduction, and by elimination of all types of breeding sites of the vector species under domestic and peridomestic situations, through community participation supported by comprehensive legislative measures.

References
The hilly regions in India exceeding an elevation of 500 metres are known to be free of Aedes aegypti infestation. Towns below this altitude in the foothills of the Himalayas show a very scanty population of the vector\(^1\). In the wake of the DHF outbreak in Delhi in 1996\(^2\), Haldwani town, situated in the foothills of the Kumoan hills in the western Himalayas, reported two cases of dengue fever in 1996. Alarmed by this incidence, a rapid survey of Aedes aegypti, the vector of DF/DHF, was undertaken in Haldwani to determine the receptivity of the town to DF/DHF during the peak of the rainy season in August-September 1997.

The studies were restricted to four urban localities, namely, Gurunanakpur, Gobindpura, Subash Nagar and Avas-Vikas Colony. The results of the survey are given in Table 1.

Table 1. Results of Aedes aegypti survey in Haldwani town during August-September 1997

<table>
<thead>
<tr>
<th>Locality</th>
<th>House Imp.</th>
<th>Gurunanak-pura</th>
<th>Gobind-pura</th>
<th>Subash Nagar</th>
<th>Avas-Vikas Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>House Index (HI)</td>
<td>-</td>
<td>51.3</td>
<td>64.2</td>
<td>22.6</td>
<td>8.7</td>
</tr>
<tr>
<td>Container Index (CI)</td>
<td>-</td>
<td>48.8</td>
<td>54.5</td>
<td>16.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Breteau Index (BI)</td>
<td>-</td>
<td>143.5</td>
<td>191.00</td>
<td>47.9</td>
<td>11.2</td>
</tr>
</tbody>
</table>

The highest larval indices were recorded in Gobindpura, a central locality, and followed by Gurunanakpura, Subash Nagar and Avas-Vikas Colony, the peripheral-most area. The highest level of breeding was detected in old/used tyres (51.5%), followed by evaporation coolers (41.8%), pitcher mudpots (34.60%), drums...
and buckets (22.8%), discarded bottles (19.1%), cement tanks (14.5%), and flower pots (4.1%).

The findings highlighted that the town maintains a high receptivity level for DHF, as it is an important rail and road-head for the Kumoan hills and is prone to high volumes of traffic (both men and material) from areas in the plains with endemic DF/DHF.

**Acknowledgements**

The authors gratefully thank Dr Sarala Subbarao, Director, Malaria Research Centre, Delhi for constant encouragement and guidance. They also thank Mr C.S. Mehra, Mr C.S. Bisht, Mr M.C. Sharma, Mr H.S. Negi, Mr D.C. Joshi, Mr Ram Kishore, Mr R.P. Prashad and Mr H. Gupta for technical assistance, and Mr H.C. Pandey for typing the manuscript.

**References**

Prevention and Control of Dengue and Dengue Haemorrhagic Fever - Comprehensive Guidelines

WHO Regional Publication, SEARO NO. 29, 1999
WHO/SEARO, NEW DELHI
Pages: 134
Price: Indian Rs. 300

Dengue fever is known to be endemic in countries of the WHO South-East Asia Region. However, the post-World War II period experienced an increased frequency of dengue epidemics, particularly the emergence of its severe forms - dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). This was triggered by varied kinds of developmental activities, increasing urbanization, migration and travel promoted by the opening up of economies and globalization. These activities resulted in a high build-up of the breeding potential of the vector populations on the one hand and the spread of all the serotypes of the dengue virus on the other. Today, seven of the 10 countries in the Region (with the exception of Bhutan, DPR Korea and Nepal) are hyperendemic and the morbidity ranges from 10,000 cases to 1.5 million cases, with the case-fatality rate ranging from 0.5% to 5%.

The WHO South-East Asia Region represents two climatic zones, namely, arid to deciduous wet and tropical monsoon zones. It has been observed that different geographical areas show a variable response to the infection and, accordingly, present different epidemiological patterns. The complex epidemiology of DF/DHF gets further modified at local level by different socio-economic and socio-cultural practices. These epidemiological complexities call for specific solutions for each geographical area.

The present guidelines try to meet the specific requirements of the countries in the Region, as outlined in the Regional Strategy for Prevention and Control of DF/DHF. The guidelines comprise 14 chapters and a similar number of annexures.

Different chapters provide comprehensive and updated information on the epidemiology of DF/DHF; clinical manifestation and diagnosis; clinical management of DF/DHF; laboratory diagnosis; epidemiological surveillance; vector distribution and bioecology; prevention and control of DF/DHF; sustainable prevention and control,
evaluation of control measures; Regional Strategy for DF/DHF control; and emergency preparedness and effective response. The last two chapters list WHO-supported activities and extensive bibliography for further reading. The annexures provide detailed information on the operational aspects of diagnostic tests and other interpretations – vector identification, vector surveillance, chemical control and other community actions.

These guidelines offer step-by-step actions with comprehensive information in a most simple language. The guidelines are a “must read” for clinicians, laboratory experts, programme managers and researchers alike.
Dengue fever (DF) dengue haemorrhagic fever (DHF) has emerged as a major tropical viral disease. It is estimated that there are between 50-100 million cases of DF and about 500,000 cases of DHF each year which require hospitalization. Over the last 10-15 years in countries in the WHO South-East Asia Region, DF/DHF has spread to rural areas as well, where clinical diagnosis and management facilities are lacking, either by way of skills or in essential equipment/blood products under the primary health care system. Consequently, by the time the patient is referred to the tertiary level of health care, it is already too late to save his life.

Attempts have been made by countries of the Region to strengthen the secondary level of health care, viz. town/district hospital and community health centre/sub-district hospital, where hospital admissions including facilities for intravenous fluids and blood transfusions are available. These small hospitals have been equipped with essential drugs, blood hematocrit and other blood products in a bid to handle at least uncomplicated DHF cases to reduce the workload at the tertiary level of health care.

The present guidelines for the treatment of DF/DHF are intended to help staff working in small hospitals primarily to treat uncomplicated cases of DF/DHF. However, if some patients develop complications and it is not feasible to refer them to a bigger hospital, then the guidelines given in this document should be followed to provide intensive care.

For simplification, the guidelines cover 12 topics, starting with the manifestation of dengue infection, recognition of DF/DHF, disease course, grading the severity of dengue infection, treatment of DF/DHF, fluids required for intravenous therapy, important instructions for the treatment of DHF, dos and don’ts, signs of recovery, criteria for discharging patients, and reporting. The guidelines provide further information in three annexures on drawing blood samples and their storage, hand-out for patients’ parents/family members and, finally, information on personal protection against DF/DHF.

The guidelines are a “must read” for health care providers at the secondary level to help them reduce mortality due to DHF.
Strengthening Implementation of the Global Strategy for Dengue Fever/
Dengue Haemorrhagic Fever Prevention and Control

(WHO/CDS/(DEN)/IC/2000.1)

WHO, in collaboration with the United States Agency for International Development (USAID), convened a meeting in Geneva from 18 to 20 October 1999 to consider ways of strengthening the implementation of the global strategy for the prevention and control of dengue/dengue haemorrhagic fever, which was developed in 1995 (“Key Issues in Dengue Vector Control Toward the Operationalization of a Global Strategy”) (CTD/FIL(DEN)/IC/96.1). The meeting was attended by over 30 scientists with public health expertise in dengue and other related disciplines. They included participants from Cuba, Singapore, Thailand, the Pacific Regional Vector-borne Diseases Control Project, the US Centers for Disease Control and Prevention, the International Federation of Red Cross and Red Crescent Societies, Medecins Sans Frontieres, Basic Support for Institutionalizing Child Survival, Academy for Educational Development, Queensland, the Institute of Medical Research and the Universities of London and Johns Hopkins.

The consultation was organized around three major themes:

- **Strengthening surveillance for planning and response**, including epidemiological and entomological surveillance and the monitoring of key human behaviours such as those which contribute to the availability of mosquito larval habitats;
- **Reducing the disease burden** through accelerated training and adoption of WHO standard clinical management guidelines for DHF; and improving emergency preparedness and response; and
- **Changing behaviours** through the development of a package of tools, approaches and guidelines for sustainable prevention and control that will address the problem at the individual, household, community, institutional and political levels as well as foster intra- and inter-sectoral partnerships for programme implementation.

The main issues and recommendations arising from the meeting were that:

- There was an urgent need to standardize and strengthen disease surveillance systems and to use the information more effectively for operational planning. With strong
systems, early indications of impending epidemics can provide as much ‘lead time’ as possible to mount an effective response, both in terms of organizing health care facilities and for implementing mosquito control measures. Improved methods of monitoring vector populations were also needed.

• The case-fatality rates of dengue haemorrhagic fever could be substantially reduced through the adoption of standard clinical management practices (Dengue haemorrhagic fever. Diagnosis, treatment, prevention and control, 2nd edition, WHO Geneva, 1997). Hence, there should be an acceleration of capacity-building and clinical management training in countries where DHF is endemic and/or epidemic, with a view to reducing the case-fatality rates to <1%.

• In addition to the issues of quality of care in government and private health facilities, attention should be given to the understanding of disease recognition and care in the home, particularly for children, and treatment-seeking behaviours of the care-providers (frequently patients arrive at referral hospitals when they are already in a critical condition and when it may be too late to save them). For dengue, this is an area that has received little attention, but is one which offers promise for further reducing the disease severity and mortality.

• There are serious limitations in the capacity of vector control interventions to reduce or interrupt dengue transmission during epidemics. In order to strengthen vector control guidelines, further evaluation of available measures and research on potential new interventions are considered a priority.

• Behavioural change interventions to reduce the availability of breeding sites of the vector mosquito are central to community-based dengue prevention and control programmes. However, most programmes focus mainly on improving ‘knowledge’ about the disease and its control rather than on the ‘practices’ or behaviours that contribute to the problem. As with treatment-seeking, an understanding of the key behaviours and a comprehensive approach to behaviour change is needed in order to achieve greater success and sustainability.

• Whereas ministries of public health have traditionally shouldered much of the responsibility for dengue control, effective and sustainable control requires new partnerships to be forged within the public sector, and between the public and commercial sectors, which can meet both enterprise and public health objectives. For purposes of advocacy, country- or municipal-level model projects should be established in several countries.
The purpose of this visit by the External Review Team was to review the current dengue/dengue haemorrhagic fever (DF/DHF) prevention and control programme in Thailand and to provide technical advice on developing a new National Dengue Prevention and Control Plan (NDPCP). The terms of reference were to:

1. Review the situation of dengue/DHF and determine the factors associated with the epidemic and geographical spread in the country;

2. Review the overall dengue prevention and control programme, including policies, strategies, infrastructure and programme delivery at provincial, municipality, district and community levels;

3. Review the management and administrative aspects and logistics, as well as to identify problems and constraints encountered in implementing the dengue prevention and control programme through the general health service system, and

4. Make recommendations for improving policies and strategies, including resource mobilization and establishment of a network of partners, to control dengue in the country.

The Team visited organizations, agencies, institutions and groups responsible for or involved in DF/DHF prevention and control in Thailand, critically reviewed approaches, methods, procedures, epidemiological and economic data and outcomes, and had intensive discussions on the approaches that may have the greatest chance of success in Thailand. It was concluded that the primary emphasis of the NDPCP should be focused on the large urban centres of the country using an integrated, community-based approach to *Aedes aegypti* control by larval source reduction. This will require strong partnerships between the Ministry of Public Health (MoPH) and provincial, city, district and village-level health authorities, and with
other civic and private organizations in the community. The recommendations included:

(1) The NDPCP should include the following five basic components: (a) surveillance; (b) emergency response; (c) clinical diagnosis and case management; (d) community-based, integrated mosquito control, and (e) research.

(2) The roles and responsibilities at each level of programme structure must be clearly identified and strengthened through capacity-building.

(3) The surveillance system should include both passive and active components, the latter being laboratory-based and focused on early warning predictive capability for epidemic transmission.

(4) The emergency response capability should emphasize larval source reduction and should be responsive to both seasonal and epidemiological indicators of increased transmission.

(5) There should be continued and more intensive emphasis placed on training physicians, nurses and paramedics in clinical diagnosis and management of DHF.

(6) Detailed guidelines on integrated vector control should be drafted and provided to all provincial, district and local health offices. These guidelines should emphasize vector control through sustainable environmental management and de-emphasize reliance on insecticides.

(7) A national training plan for *Aedes aegypti* control should be developed and implemented to ensure that trainees have the technical, communications and teaching skills to implement the NDPCP.

(8) A concerted effort must be made to control the many productive mosquito larval habitats in urban and suburban areas such as businesses, tyre shops, vacant lots, etc. that do not fall within the jurisdiction of the government and volunteer health workers.

(9) A core group of experts in social and behavioural sciences should be formalized to advise the MoPH on developing and utilizing educational materials and programmes to create community ownership of the NDPCP. This group should also guide efforts to evaluate these materials and programmes.

(10) Direct communication links should be established between the National Office of Dengue Control, Vector-Borne Disease Control Offices and the provincial and district health teams to ensure continuity of message production and dissemination.

(11) In order to ensure broad intersectoral collaboration and support from the private sector, an
NGO such as Rotary International should be represented on the National Dengue Prevention and Control Committee. The roles and responsibilities at each level of programme structure must be clearly identified and strengthened through capacity-building.

(12) The MoPH should develop a management plan for dengue prevention and control that clearly designates lines of authority for policy decisions and supervision and for communication and coordination.

(13) The CDC of the MOPH should play a more prominent leadership role in the implementation of the NDPCP. This can be achieved by developing greater policy, advocacy and intersectoral collaboration at the national level, actively providing technical support to develop integrated mosquito control programmes and by focusing funds from Japanese loans on developing a sustainable social mobilization and awareness plan.

(14) The MoPH should develop and implement a new extramural research programme to gather information needed to strengthen the implementation of the NDPCP.
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:


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.
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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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