# SILENT TRANSMISSION OF VIRUS DURING A DENGUE EPIDEMIC, NAKHON PATHOM PROVINCE, THAILAND 2001

Thaval Poblap[1], Narong Nitatpattana[2], Aree Chaimarin[1], Philippe Barbazan[2,3], Gilles Chauvancy[2,3], Sutee Yoksan[2] and Jean-Paul Gonzalez[2,3]

[1]Nakhon Pathom Provincial Health Office, Ministry of Public Health, Nakhon Pathom; [2]Center for Vaccine Development-Research Center for Emerging Viral Diseases, Institute of Science and Technology for Research and Development, Mahidol University, Nakhon Pathom, Thailand; [3]Institut de Recherche pour le Developpement, IRD Ur034, Paris, France

Abstract. In the year 2001 a large dengue fever (DF)/dengue hemorrhagic fever (DHF) outbreak occurred in Nakhon Pathom Province, Thailand. Three thousand one hundred twelve cases of DHF were reported, an attack rate of 393 per 100,000 population. The Nakhon Pathom Provincial Health Office immediately carried out a control action according to WHO recommendations. Active serological surveys and viral RNA isolation were carried out to detect silent transmission of dengue virus in 329 healthy volunteers in Nakhon Pathom Province subdistricts where the dengue epidemic had the highest rate of infection of 2.5 per 1,000. Eight point eight percent of these volunteers had a serum sample positive for DF/DHF virus IgM antibody. The highest prevalence occurred in the 15 to 40 year old group. In two instances viral RNA was detected by PCR and dengue serotype 3 was subsequently identified. The data support the hypothesis of subclinical infection with dengue virus. This high frequency of virus circulation combined with a high population density, urbanization and increasing breeding sites for mosquitoes, needs to be taken into account in the evaluation of viral transmission during and after epidemics. This underlines the importance of community-based control in informing people of their involvement in virus transmission and the importance of personal protection.

## INTRODUCTION

Dengue virus belongs to the Flaviviridae family. Dengue virus has 4 serotypes responsible for a growing health problem in Asia, Africa, Central and South America (Gubler, 1997. Dengue is a viral disease with clinical features that vary in intensity according to host and viral strain characteristics. Dengue hemorrhagic fever (DHF) is a rare clinical entity for the majority of physicians in North America. There is a need for information regarding the clinical and epidemiological features of DF/DHF diagnosis, therapy and control. The clinical spectrum of the disease ranges from asymptomatic to severe hemorrhagic forms. Most of the clinical descriptions explores the physiological mechanisms involved in the pathogenesis of DHF (George and Lum, 1997; Gubler, 1997).

In Thailand, during the 1987 DF/DHF epidemic, 87% of dengue infections in children were asymptomatic (Burke *et al*, 1988). The epidemic involved several factors but virus transmission played a central role. Patients with severe clinical syndrome are usually isolated by hospital barriers, others are exposed to mosquito bites during viremia.

This study evaluated the present work aims to present some insight and the risk factors for virus transmission, especially in

Correspondence: J-P Gonzalez, Center for Vaccine Development-Research Center for Emerging Viral Diseases, IRD Mahidol University, Nakhon Pathom Province 73170, Thailand.
Tel/Fax: +66 (0)2441-0189
E-mail: frjpg@mahidol.ac.th

asymptomatic infected persons.

## MATERIALS AND METHODS

### Study design

A cross-sectional study took place from March 27 to April 11, 2001 during a dengue fever/dengue hemorrhagic fever (DF/DHF) outbreak. Human paired serum samples (7 days apart) were obtained from healthy volunteers from a rural area of Nakhon Pathom Province.

### Study population and sites

Nakhon Pathom Province encompasses an area of 2,168 km$^2$ within the Central Plain region of Thailand with a population of 791,903 persons (Fig 1).

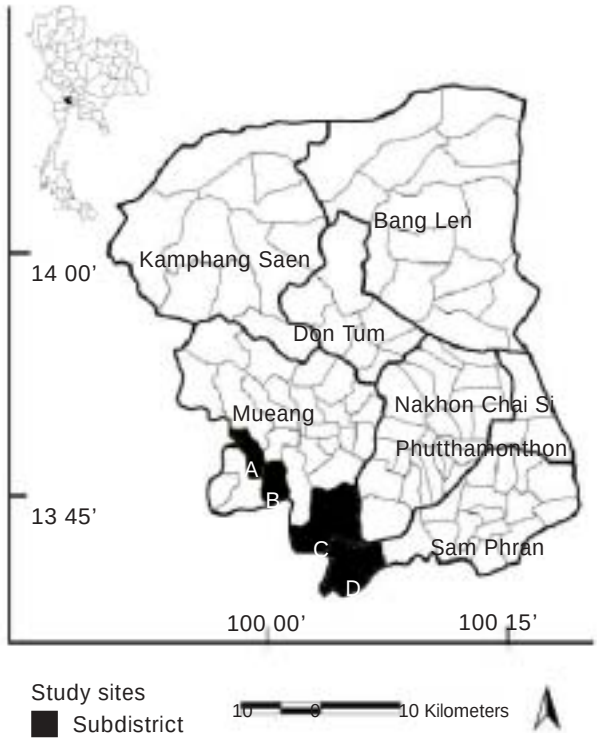


Fig 1–Study sites in Nakhon Pathom Province subdistricts: A Nhong Din Dang, B Wang Yen, C Don Yai Hom in Mueang district and subdistrict D Talad Ginda in Sam Phran district.

Within the Nakhon Pathom Province, 15 villages belonging to four subdistricts of the Mueang and Sam Phran districts were investigated. In the Mueang district, the surveyed villages (administratively named as Mooban) were distributed over the three subdistricts: Mueang Don Yai Hom, Mooban 1 to 4, 6, 7, 9 with a total population of 11,227 inhabitants; Nong Din Daeng subsdistrict, Mooban 2 to 5 and 8 with a total population of 8,239 persons; Wang Yen subsdistrict, Mooban 1 and 5 with a total population of 2,818 persons. In the Sam Phran district, the survey was conducted in Mooban 3 of the Talad Ginda subdistrict, with a total population of 4,269 persons.

### Case definition

Villages were randomly chosen within the epidemic districts using a simple random sampling without replacement. Announcement of the survey was posted and healthy villagers who volunteered were invited to participate in the interview. All volunteers were included and tested without any exclusion criteria.

All necessary data were collected by interview, including any clinical history of fever.

### Data collection

DF/DHF patients from the hospital were reported using Epidemiology form 506 to the Nakhon Pathom Provincial Health Office, Ministry of Public Health, Nakhon Pathom Province, Thailand.

### Serum collection and transportation

After collection, the human sera were kept at 4ºC in an ice chest for less than two hours until transportation to the laboratory. All the sera were then centrifuged, decanted, and stored at -80ºC in the laboratory until testing.

### Serology

ELISA tests for serology were carried out according to the MAC-ELISA test routinely used at the Research Center for Emerging Viral Diseases following previously described

techniques (Innis *et al*, 1989). Sera were diluted to 1:100 and tested for IgM and IgG using a mix of dengue: Dengue-1 (Hawaii), Dengue-2 (Tr 1751), Dengue-3 (H871), Dengue-4 (H241) and JE (Jagar#01) antigens.

Viral RNA detection

For each sample, 100 µl of sera was used for RNA extraction using 1 ml of Trizol (GIBCO-BRL, Gaithersburg). A consensus DGS2 primer (5'-TTg CAC CAA CAg TCA ATg TCT TCA ggT TC-3') was used for priming viral RNA for cDNA synthesis reaction (Deubel *et al*, 1990). A nested RT-PCR was then performed following the previously described method by Lanciotti *et al* (1992) using the DGS2 and a DGS1 (5'-TCA ATA TgC TgA AAC gCg CgA gAA ACC g-3') primer. The reverse primer, specific for 1 to 4 dengue serotypes (TS1 -5'-CgT CTC AgT gAT CCg ggg g-3', TS2 -5'-CgC CAC AAg ggC CAT gAA CAg-3', TS3 -5'-TAA CAT CAT CAT gAg ACA gAg C-3', TS4 -5'-CTC TgT TgT CTT AAA CAA gAg A-3') were used for a second and final type identification PCR previously described (Deubel *et al*, 1990).

## RESULTS

A total of 329 human serum specimens from the Nakhon Pathom Province were collected and tested. Twenty-nine sera tested positive for dengue IgM (8.8%) and one for Japanese encephalitis IgM. Some of them reported having had a history of fever with the week before testing. Thirteen sera tested positive for IgG flavivirus antibody (4.0%). Two hundred eighty-six serum samples had no flavivirus antibody; of these 17% (56/329) reported a fever ending more than a week prior to sampling (Table 1). Most of the serologically positive persons (80.8%; 42/52) reported no previous fever (Table 2).

The IgM percentage rates were 15.4% in Wang Yen, 12% in Talad Ginda, 9.3% in Don Yai Hom and in 5.1% Nong Din Daeng (Table 3).

Table 1
Flavivirus IgG/IgM antibodies (ELISA) among a selected normal population during a March-April 2001 dengue epidemic in Nakhon Pathom Province, Thailand.

| Antigen[a] | Positive (%) |
|---|---|
| Dengue | 29 (8.8) |
| JE | 1 (0.3) |
| Flavivirus | 13 (4.0) |
| Total positive | 43 (13.1) |
| Negative | 286 (86.9) |
| Total tested | 329 |

[a] = Virus specific antigen detection was determined by WHO criteria, an IgG/IgM OD value rate.

Table 2
Flavivirus reacting IgG/IgM antibodies (ELISA) among a selected population during the March-April 2001 dengue epidemic in Nakhon Pathom province, Thailand.

| Antigen | No clinical record[a] | Past fever history[a] |
|---|---|---|
| Dengue | 29/35 (82.9) | 6/35 (17.1) |
| JE | 0/1 (0.0) | 1/1 (100.0) |
| Flavivirus | 13/16 (81.3) | 3/16 (18.7) |
| Total | 42/52 (80.8) | 7/52 (19.2) |

[a] = Positive/total tested (%)

The highest rate (20.0%) of those with dengue IgM antibodies was in the 40-49 year old age group (3/15), followed by 17.4% (4/23) and 11.1% (2/18) in the 15-19 and 20-29 year old age groups, respectively (Table 4).

Of those positive for dengue IgM antibody 13.7% (23/168) were males and 7.5% (12/161) were females, showing no significant difference between the two.

Of the 329 samples tested for viral RNA,

Table 3
Flavivirus reacting IgM antibodies (ELISA) among a selected normal population without symptoms of infection and, hospitalized patients clinically diagnosed as dengue hemorrhagic fever during the 2001 dengue epidemic in Nakhon pathom Province, Thailand.

| Subdistrict | IgM positive[a] |
|---|---|
| Don Yai Hom | 19/204 (9.3) |
| Nong Din Daeng | 4/79 (5.1) |
| Wang Yen | 2/13 (15.4) |
| Talad Ginda | 4/33 (12.1) |
| Total | 29/329 (8.8) |

[a]= positive/total tested (percentage)

Table 4
Flavivirus reacting IgM antibodies (ELISA) among a selected normal population, and those clinically diagnosed with dengue fever/dengue hemorrhagic fever (DF/DHF), March-April 2001, during dengue epidemic of Nakhon Pathom Province, Thailand.

| Age | Serosurvey[a, b] | Clinical survey[b] |
|---|---|---|
| 0-4 | 1/22 (4.6) | 2/1,563 (0.1) |
| 5-9 | 10/112 (8.9) | 10/2,134 (0.5) |
| 10-14 | 4/96 (4.2) | 20/1,987 (1.0) |
| 15-19 | 4/23 (17.4) | 17/2,026 (0.8) |
| 20-29 | 2/18 (11.1) | 5/4,584 (0.1) |
| 30-39 | 1/14 (7.1) | 3/4,784 (0.1) |
| 40-49 | 3/15 (20.0) | 0/3,830 (0.0) |
| >50 | 2/23 (8.7) | 0/5,642 (0.0) |
| Total | 29/329 (8.8) | 57/26,553 (0.2) |

[a] = Clinical diagnosis from hospital
[b] = Number of positive / total tested (percentage)

dengue serotype 3 was identified in two instances (0.6%) in specimens from Don Yai Hom subdistrict. One of these reported a history fever.

## DISCUSSION

Mac ELISA for IgM detection is useful for follow-up of active virus circulation, including clinical cases and silent transmission by surveying the dengue IgM positively rate. Serology reflect dengue incidence and can be consider a good marker of epidemic dynamics (Nisalak *et al*, 2003). During the epidemic, asymptomatic cases were 20 to 60 fold greater at the district levels than those hospitalized cases of severe dengue infection.

Asymptomatic dengue virus transmission cannot be underestimated. These contribute to dengue incidence despite classical control measures.

An important outbreak occurred in Nakhon Pathom Province, and despite systematic vector control the number of dengue cases increased after the beginning of the outbreak. Although the highest incidence was found in the 5 to 19 year old age group (Gluber 1997), silent transmission occurs more commonly in the 15 to 40 year old group. No differences in gender were observed with silent transmission, similar to the those with clinical manifestations of dengue virus infection (Chareonsook *et al*, 1999).

The two cases of dengue 3 viral RNA detected confirmed dengue 3 serotype was the main circulating serotype and responsible for silent transmission in the epidemic area.

Persons with sub-clinical dengue infection contribute to the dengue epidemic. In community based control, health information should be given regarding the high risk of silent dengue virus infection.

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