# Dengue Haemorrhagic Fever in the South of Vietnam during 1975-1992 and Its Control Strategy

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**Abstract:** The incidence of dengue (DEN) virus infection in Vietnam has increased dramatically in the past 30 years. From 1960 up to now, the number of dengue haemorrhagic fever (DHF) cases has been continuing to increase and recorded as the greatest one in Southeast Asia and the Western Pacific Regions.

DHF was endemic in South Vietnam and 30–380 cases/100,000 population were reported annually. Major epidemics have occurred in a 3–4 years frequency, as in 1975, 1978–1979, 1983 and 1987. The 1987 outbreak was the largest one with 83,905 cases and 904 deaths. DHF epidemics usually took place from June to November every year. The peak transmission was recorded in July-August and September, coinciding with the rainy season and the breeding period of *Aedes aegypti*. The majority of confirmed cases was children of 5–6 year-old-group, in which there was no sex difference.

In the virological surveillance in Ho Chi Minh City and in some surrounding provinces, several DEN virus strains were isolated from patients' blood as well as from the mosquitoes. In the 1987 DHF outbreak, DEN-2 was the dominant sero—type (90.5%). But from 1990 the epidemic sero—type has changed, DEN-1 was introduced and was continuing to grow—up (58%). Then in 1992 outbreak, the DEN-2 was reintroduced, from 26.3% in 1991 has increased to 41.4%. Serological investigation of healthy persons in 12/17 southern provinces had demonstrated that there were relatively wide circulations of DEN, Japanese encephalitis (JE) and chikungunya (Chik) viruses and the endemic strains of DEN were found different in some studied areas. There had been augmentation of primary infection rate and decrease of the susceptibility rate in children prior to the DHF epidemic. Seven JE virus strains were isolated from dengue fever (DF) patients' blood. This fact has introduced as a new concept in the isolation of JE virus.

In the final part of the report, the authors stressed on the "Active surveillance" as a new strategy for controlling the DHF epidemic in South Vietnam. The goal of this active surveillance is to have early warning and predictive capability for epidemic dengue, based on serological and clinical surveillance. Because dengue cases were detected in January—February of every year, so the base line is: when first cases of DF/DHF were detected, it needs to be promptly implemented the larval control and insecticide must be sprayed around the patient's houses. But now almost Provincial Centers of Hygiene and Epidemiology often waited and when big DHF epidemic had already occurred, they started to implement its control measures, so it is too late to control the DHF outbreak.

Key words: Dengue haemorrhagic fever, Vietnam.

#### INTRODUCTION

DHF and dengue shock syndrome (DSS) have now become a major public health problem in Vietnam. From 1960 up to now DHF epidemics tend to spread widely with continuous increase in number of patients and deaths (Do et al., 1989). In Hanoi Vietnam, a small and limited DF was first described in 1958, then in 1969 the first outbreak of DHF occurred in 19/25 provinces in the North (Doan et al., 1970; 1971). In South Vietnam, the disease first appeared sporadically in 1960 with about 60 children died. Thereafter in 1963, an outbreak occurred at Cai Be, Chau Doc, Hong Ngu, Tan Chau and Cao Lanh with 331 children were hospitalized, 116 of them died (Nguyen et al., 1971).

According to the statistics of the WHO (Umenai and Suzuki, 1990) from 1963 to 1988 the DHF patients and fatal cases in Vietnam were 1,111,734/10,415. This was the greatest patient number in comparison with other countries in Southeast Asia and the Western Pacific Regions. From 1963 to 1992 in South Vietnam there were 518,332 patients with 8,958 deaths and the number of DHF patients ranked only after diarrheal diseases (Do *et al.*, 1990; 1991).

# MATERIALS AND METHODS

# Epidemiological, entomological and clinical data

The epidemiological and entomological data were provided by Pasteur Institute Ho Chi Minh City. The clinical records were collected from the Center of Tropical Diseases, the Pediatric Hospitals No 1 and No 2 in Ho Chi Minh City.

#### Specimens

Blood samples were collected by venipuncture from children within 1-4 days after the onset of illness. The blood was taken aseptically, agitated in bottle with glass beads, then centrifuged, the serum was withdrawn for viral isolation. Mosquito specimens, after identification, were kept for blood digestion, triturated with PBS pH 7.8, containing 0.4% bovine albumin, then centrifuged. The mid whitish liquid portion was pipetted out, filtered through Sartorius GmbH-3.400 membrane (Göttingen, Germany) then used for viral isolation.

#### Viral isolation

From 1978 to 1986, blood samples were processed in suckling mice. From 1982, in *Toxorhynchites splendens* (Rosen and Gubler, 1974) and from 1987 up to now, the C6/36 and TRA-284SF (Igarashi, 1978; Kuno *et al.*, 1985) cell cultures were used for virus isolation. For identification of the isolates, the neutralization test in suckling mice was used. Then from 1987 the direct immunofluorescent antibody test (DFA) and the indirect immunofluorescent antibody test (IFA) with specific monoclonal antibodies (MnAb) (Henchal *et al.*, 1985) have been performed.

# Serological studies

Serum samples for diagnosis were obtained by venipuncture from DHF/DSS patients in the Government Hospitals and Provincial Centers of Hygiene and Epidemiology. The first serum was taken on the day of admission and the second serum, on 3–5 days later. Blood samples for serological survey were collected in 12/17 provinces. Blood was taken from the finger tip through a lancet puncture, then collected into capillary tube. All specimens were obtained at random in different age groups. Serum specimens are tested by the micro haemagglutination—inhibition (HI) test (Clarke and Casals, 1958) using DEN, JE and Chik antigens. The IgM capture ELISA (MAC ELISA) (Kuno *et al.*, 1987) has been used since 1987. Results were interpreted according to the standard outlined of the WHO meeting on DHF 1980 (Technical Advisory Committee on DHF, 1980).

# Virus strains

Following virus strains were used: DEN-1 (ATCC. VR. 71), Hawaiian, Mochizuki; DEN-2 (New Guinea C. VR. 222); DEN-3 (H87. VR. 216); DEN-4 (ATCC. VR. 217 H241); Chik (IPDA/CS 13); JE (Nakayama).

### The biological products

The hyper-immune mouse ascitic fluids (HIMAF) of 4 DEN types were produced at Yale Arbovirus Research Unit (WHO International Reference Center) and the National Institute of Allergy and Infectious Diseases Bethesda Maryland USA. The MnAb DEN-1 Hawaii 15F3-1-15 and D2-1F1-3, DEN-2 New Guinea C 3H5-1-21, DEN-3 H 87 5D4-11-24 and DEN-4 H 241 1H10-6-7 were produced at CDC Atlanta Georgia USA. The peroxidase conjugated anti-flavivirus human IgG was produced at CDC Atlanta Georgia USA. The anti-flavivirus human IgG-FITC conjugate was produced at CDC Puerto Rico USA and in Pasteur Institute Ho Chi Minh City. The anti-mouse IgG-FITC conjugates were produced by Sigma Chem. Co. St. Louis, MO USA and Pasteur Paris Production. The anti-human IgM ( $\mu$ -chain specific) goat IgG was produced by Cappel Laboratories Pa USA. The hyperimmune sera of JE and Chik were produced in our laboratory, using white mice weighed 18-20 g with viral strains mentioned above.

# RESULTS AND DISCUSSION

# The epidemiological data

The number of DHF patients and deaths by years were summarized in Tables 1 and 2. In the first decade (Nguyen *et al.*, 1971) DHF epidemics have occurred only in crowded population, then year by year the outbreaks trend to develop more largely. From 1975, the DHF outbreaks trend to overwhelm in 16 provinces except the mountainous region. During 1975–1981 (Do *et al.*, 1983) in every year Ho Chi Minh City had the highest number of patients, but from 1982 this situation was transferred to 10 other provinces such as Tay Ninh, Song Be, Lam Dong, Dong Nai, Vung Tau, Long An, Tien Giang, Ben Tre, Kien Giang and

Minh Hai. Since 1963, large DHF epidemics have occurred every 3 to 4 years with a consistent increase in both prevalence of cases and deaths (Do *et al.*, 1992). In 1988 the first large DHF outbreak occurred at Bao Loc—Lam Dong which is a mountainous region and since then the DEN activities have occurred in all provinces in South Vietnam (Do *et al.*, 1990). Large DHF epidemics in South Vietnam in 1975, 1979, 1983, 1987 occurred at the same time with big epidemics in the whole country (Do *et al.*, 1992).

Table 1. Number of DHF patients and deaths by years

	Occurrence of DHF/DSS in South Vietnam by year								
Year	Reported cases (deaths)	Year	Reported cases (deaths)						
1963	331 (116)	1969	680 (44)						
1964	1,168 (172)	1970	257 ( 37)						
1965	300 ( 37)	1971	174 ( 27)						
1966	607 (101)	1972	1,338 (210)						
1967	102 ( ?)	1973	4,368 (283)						
1968	521 ( 19)	1974	3,930 (404)						

From 1963 to 1971 only the in-patients were recorded.

**Table 2.** Reported DHF/DSS cases and deaths in South Vietnam during 1975-1992

37	Мо	orbidity	М	ortality	CFR
Year	No.	Rate/10 <sup>5</sup>	No.	Rate/10 <sup>5</sup>	%
1975	19, 416	112.44	397	2.29	2.04
1976	6, 151	35.62	558	3.23	9.07
1977	8,362	47.33	447	2.53	5.34
1978	17,055	94.37	484	2.67	2.83
1979	21, 285	115.13	466	2.52	2.18
1980	11,997	63.43	500	2.64	4.16
1981	5,933	30.66	76	0.39	1.28
1982	11, 281	56.99	155	0.78	1.37
1983	77,087	380.73	1,301	6.42	1.68
1984	14,562	70.30	286	1.38	1.96
1985	19,927	94.04	209	0.98	1.04
1986	15,647	72.18	243	1.12	1.55
1987	83,905	378.37	904	4.07	1.07
1988	49, 237	217.04	587	2.58	1.19
1989	18,923	83.09	178	0.78	0.94
1990	41,517	174.87	298	1.25	0.71
1991	39,917	168.36	244	1.03	0.61
1992	42,330	174.61	173	0.71	0.40

# The seasonal fluctuation in DEN activities in South Vietnam

From 1975 to 1992, the number of DHF patients was recorded all year round but the transmission increased substantially from June to November with peak transmission in July-August or September which was closely related with rainy season—a breeding period of mosquito vector (Do *et al.*, 1992). It is noted that a lot of DHF patients were recorded in every first trimester. From 1975 to 1982 (Do *et al.*, 1983), depending on each year an approximate number of 400 to 2,000 patients was recorded in January. During 1983—1990 the mean morbidity and lethality was 1,048/10 in January, 716/10 in February, 575/7 in March and 780/14 in April (see Fig. 1). These patients with overt diseases represented only the tip of the DHF iceberg protruding above the water (Gubler, 1987).

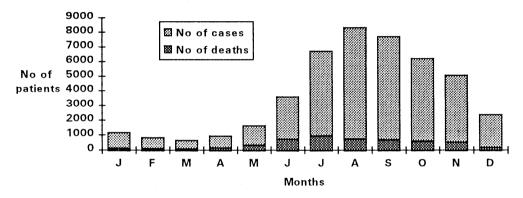


Fig. 1. Monthly distribution of the mean number of DHF patients and fatal cases in South Vietnam, 1983-1990.

#### Age distribution

During 1978-1992, based only on laboratory confirmed cases, the number of patients suffering from DHF essentially consisted of children under 9 years old. The age subject to highest morbidity was 5-6 year-old-group, with fewer cases occurring in adults (2.75% of DHF patients, in 1990) (Do *et al.*, 1992). Contrary to the South, the DHF morbidity in adults in the North of Vietnam was high (53.12% in the 1983 epidemic). This is a meaningful difference between the 2 regions (Do *et al.*, 1985).

# Sex distribution of laboratory confirmed cases

In 1979, 1983, 1987 large epidemics, the male: female mean ratio was 0.942/1, but in 11 intercalary smaller epidemics (excepted 1988) the male: female mean ratio was 1.017/1. The difference in sex attacked by DHF in 3 large outbreaks (1979, 1983, 1987) compared with the remaining ones had no statistic significance ( $\chi^2=1.99$  with P>5%). Thus, in South Vietnam the sex was not a risk factor for DHF/DSS (Do *et al.*, 1992).

# Some climatic and entomological data

The mean temperature in Ho Chi Minh City during 1983-1990 was  $27.4^{\circ}$ C, the difference between the rainy and dry season did not exceed  $3.3^{\circ}$ C. The relative mean humidity in 1983-1990 was 77.88%; 70.5-71.25% in dry seasons and 82.5-85% in rainy seasons. Therefore the temperature and humidity were favorable for the breeding of the mosquito population, which tended to speed up virus replication and shortened the extrinsic incubation period. The *Aedes aegypti* density, the Breteau index and the mean rainfall by month during 1983-1990 in Ho Chi Minh City were previously reported. During 1983-1990 the *Aedes aegypti* mean density was 1.02-1.30 in dry season and 1.93-2.43 in rainy season (Do *et al.*, 1992). To summarize, the climate and weather in South Vietnam allow continous breeding of mosquito population. Such factors are propitious for DEN transmission that end with DHF outbreak in every year (see Fig. 2).

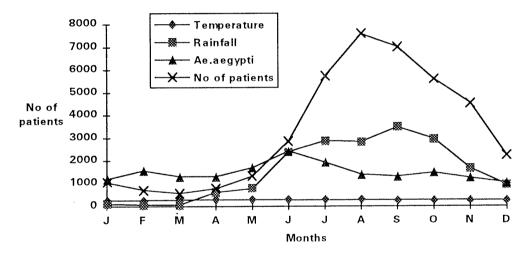


Fig. 2. The correlation between DHF epidemics with temperature, rainfall and *Aedes aegypti* density, 1983–1990.

#### Viral isolation

During 1978–1985, several DEN viruses were isolated (Do et al., 1985) but the dominant sero—type could not be identified because small number of isolates were detected and some of them were isolated in January—February. From 1987 by using C6/36 and TRA—284SF cells, the number of isolates has increased and the dominant sero—type can be established. In the largest 1987 outbreak DEN—2 was the dominant epidemic sero—type, occupying 90.5% of the isolates (Do et al., 1989). In 1988—1989, transmissions of this sero—type were documented. Then in 1990 the dominant epidemic sero—type gradually changed: from May to September, DEN—1 was introduced in Ho Chi Minh City, Long An, Vinh Long, Dong Nai with the concurrent circulation of DEN—2, this type was transmitted simultaneously throughout Ho Chi Minh City, Dong Nai, Vinh Long, Ben Tre and Dong Thap. In the queue of this epidemic, DEN—4 was detected in Cai Lay—Tien Giang (Do et

al., 1990; 1991). To summarize, the percentage of isolates was DEN-1: 58.0%, DEN-2: 32.2% and DEN-4: 9.6% (Do et al., 1993). In 1991 the ratio of the sero-type was DEN-1: 47.3%, DEN-2: 26.3%. Then in 1992 DEN-2 has been reintroduced with the ratio: DEN-1: 58.6%, DEN-2: 41.3%. Of interest is that if DEN-2 became the dominant epidemic sero-type in 1993 or 1994, a large DHF epidemic will possibly occur. Of more interest, was the fact that in 1992 one strain of DEN-1 (1F1) was isolated from a 12 years old patient diagnosed as "Encephalitis Syndrome".

Apart from the DEN viruses recovered, 7 JE viruses were isolated from patients' blood: of whom, 6 were children of 4-11 year—old group, one was a patient of 40 years old. All of them were diagnosed as DF. These results are quite interesting as compared with previous knowledge, because the isolation of JE virus from patient's blood has still been so far universally recognized as very rare in the medical literature.

# Relationships among DEN-2 isolates in sequence comparative studies and the DEN-1 sero-type specific determinants identified by monoclonal antibodies

Structural and non-structural viral antigens of DEN viruses: 29/44 strains of DEN-1 (1990: 11 strains; 1991: 4 strains; 1992: 14 strains) isolated in 1990-1992 had the same identification process as the DEN-1 strain isolated from an American after a travel in Colombia (Gubler, 1986). These strains were very difficult to identify by IFA using MnAb DEN-1 (15F3), only some cells with dull fluorescence were detected if compared with its DFA. It is possible that there was a higher ratio of virion to nonvirion antigen in the cell cultures, because the 15F3 was directed against non-structural viral antigen. Thereafter these strains were retested with the MnAb DEN-1 (1F1) and all these strains were identified as DEN-1 with clear fluorescent brilliance seen in all infected cells. Moreover in 1992 we also isolated:

- ·One strain of DEN-1 (1F1) which crossed in IFA with MnAb DEN-1 (15F3).
- One strain of DEN-1 (15F3) which crossed in 1FA with MnAb DEN-3 and DEN-4.

The conclusion is unclear, maybe this is a concurrent infection with 2 or 3 DEN viruses.

Genetic differences between strains of DEN-2 from different geographic regions: A dendrogram was constructed by calculating the similarity score obtained by sequence pair comparison. A close relationship has demonstrated between the 4 strains of DEN-2/Vietnam/87 with the Caribbean topotype (Jamaica 1983) isolates (Deubel *et al.*, 1993). Two other strains of DEN-2/Vietnam/87 were also sent to R. Rico-Hesse (Rico Hesse, 1990) and the results of above two studies were similar. There was a relatively close identity of these strains circulating in two regions which were very far from each other. R. Rico-Hesse has hypothesized that the DEN-2 endemic in the Caribbean basin countries has been possibly introduced into America by Cuban workers who were back home from Vietnam.

# Clinical manifestations of children from whose blood viruses were recovered

In general, patients' clinic aspect of the large 1987 DEN-2 epidemic (90.5%) was noted to be more severe than that of the 1990 outbreak (DEN-1: 58.0%), but more cases of

hepatomegaly and abdominal pain were observed than those of the 1987 outbreak (Tran, 1991).

# The serological data

The serological diagnosis was summarized in Table 3. From 1988 up to now the positive percentage of paired sera has been rather higher than those of the previous years, maybe the clinical diagnosis had much experience and by using the 2-mercaptoethanol and the MAC ELISA to detect the IgM antibody.

Table 3.	Serologic response of paired sera by HI test
	using DEN-1 antigen during 1978-1992

Year         No of sera $\%(-)$ $\%(\pm)$ $\%(+)$ 1977         811         50.8         11.1         29.1           1978         2,367         38.7         15.6         45.5           1979         2,062         38.9         14.3         46.6           1980         550         14.5         48.5         36.9           1981         661         51.7         15.2         32.9           1982         854         39.7         16.4         43.9           1983         838         31.7         15.0         53.2           1984         137         44.5         17.5         37.9           1985         105         18.0         16.1         65.7           1986         24         29.1         16.6         54.1           1987         146         45.2         13.6         41.0           1988         139         20.8         2.8         76.2           1989         37         5.4         8.1         86.4           1990         270         19.2         6.2         74.4           1991         345         27.8         0         72.2 <th></th> <th>using DEI</th> <th>ı anagan a</th> <th>uring 1010</th> <th>1000</th>		using DEI	ı anagan a	uring 1010	1000
1978         2,367         38.7         15.6         45.5           1979         2,062         38.9         14.3         46.6           1980         550         14.5         48.5         36.9           1981         661         51.7         15.2         32.9           1982         854         39.7         16.4         43.9           1983         838         31.7         15.0         53.2           1984         137         44.5         17.5         37.9           1985         105         18.0         16.1         65.7           1986         24         29.1         16.6         54.1           1987         146         45.2         13.6         41.0           1988         139         20.8         2.8         76.2           1989         37         5.4         8.1         86.4           1990         270         19.2         6.2         74.4           1991         345         27.8         0         72.2	Year	No of sera	<b>%</b> (-)	%(±)	%(+)
1979         2,062         38.9         14.3         46.6           1980         550         14.5         48.5         36.9           1981         661         51.7         15.2         32.9           1982         854         39.7         16.4         43.9           1983         838         31.7         15.0         53.2           1984         137         44.5         17.5         37.9           1985         105         18.0         16.1         65.7           1986         24         29.1         16.6         54.1           1987         146         45.2         13.6         41.0           1988         139         20.8         2.8         76.2           1989         37         5.4         8.1         86.4           1990         270         19.2         6.2         74.4           1991         345         27.8         0         72.2	1977	811	50.8	11.1	29.1
1980         550         14.5         48.5         36.9           1981         661         51.7         15.2         32.9           1982         854         39.7         16.4         43.9           1983         838         31.7         15.0         53.2           1984         137         44.5         17.5         37.9           1985         105         18.0         16.1         65.7           1986         24         29.1         16.6         54.1           1987         146         45.2         13.6         41.0           1988         139         20.8         2.8         76.2           1989         37         5.4         8.1         86.4           1990         270         19.2         6.2         74.4           1991         345         27.8         0         72.2	1978	2,367	38.7	15.6	45.5
1981       661       51.7       15.2       32.9         1982       854       39.7       16.4       43.9         1983       838       31.7       15.0       53.2         1984       137       44.5       17.5       37.9         1985       105       18.0       16.1       65.7         1986       24       29.1       16.6       54.1         1987       146       45.2       13.6       41.0         1988       139       20.8       2.8       76.2         1989       37       5.4       8.1       86.4         1990       270       19.2       6.2       74.4         1991       345       27.8       0       72.2	1979	2,062	38.9	14.3	46.6
1982       854       39.7       16.4       43.9         1983       838       31.7       15.0       53.2         1984       137       44.5       17.5       37.9         1985       105       18.0       16.1       65.7         1986       24       29.1       16.6       54.1         1987       146       45.2       13.6       41.0         1988       139       20.8       2.8       76.2         1989       37       5.4       8.1       86.4         1990       270       19.2       6.2       74.4         1991       345       27.8       0       72.2	1980	550	14.5	48.5	36.9
1983     838     31.7     15.0     53.2       1984     137     44.5     17.5     37.9       1985     105     18.0     16.1     65.7       1986     24     29.1     16.6     54.1       1987     146     45.2     13.6     41.0       1988     139     20.8     2.8     76.2       1989     37     5.4     8.1     86.4       1990     270     19.2     6.2     74.4       1991     345     27.8     0     72.2	1981	661	51.7	15.2	32.9
1984       137       44.5       17.5       37.9         1985       105       18.0       16.1       65.7         1986       24       29.1       16.6       54.1         1987       146       45.2       13.6       41.0         1988       139       20.8       2.8       76.2         1989       37       5.4       8.1       86.4         1990       270       19.2       6.2       74.4         1991       345       27.8       0       72.2	1982	854	39.7	16.4	43.9
1985     105     18.0     16.1     65.7       1986     24     29.1     16.6     54.1       1987     146     45.2     13.6     41.0       1988     139     20.8     2.8     76.2       1989     37     5.4     8.1     86.4       1990     270     19.2     6.2     74.4       1991     345     27.8     0     72.2	1983	838	31.7	15.0	53.2
1986     24     29.1     16.6     54.1       1987     146     45.2     13.6     41.0       1988     139     20.8     2.8     76.2       1989     37     5.4     8.1     86.4       1990     270     19.2     6.2     74.4       1991     345     27.8     0     72.2	1984	137	44.5	17.5	37.9
1987       146       45.2       13.6       41.0         1988       139       20.8       2.8       76.2         1989       37       5.4       8.1       86.4         1990       270       19.2       6.2       74.4         1991       345       27.8       0       72.2	1985	105	18.0	16.1	65.7
1988       139       20.8       2.8       76.2         1989       37       5.4       8.1       86.4         1990       270       19.2       6.2       74.4         1991       345       27.8       0       72.2	1986	24	29.1	16.6	54.1
1989     37     5.4     8.1     86.4       1990     270     19.2     6.2     74.4       1991     345     27.8     0     72.2	1987	146	45.2	13.6	41.0
1990     270     19.2     6.2     74.4       1991     345     27.8     0     72.2	1988	139	20.8	2.8	76.2
1991 345 27.8 0 72.2	1989	37	5.4	8.1	86.4
	1990	270	19.2	6.2	74.4
1992 43 13.9 0 86.0	1991	345	27.8	0	72.2
	1992	43	13.9	0	86.0

The sero-epidemiological study: The sero-survey of DEN, JE and Chik has been conducted for the first time in South Vietnam which are as follows (see map 1):

The cross—sectional sero—surveys in 1979 conducted in 4 localities: Ho Chi Minh City, Vung Tau, My Tho and Minh Hai were previously described (Do and Duong, 1982). Most of these 2,494 healthy persons had previously contracted DEN, JE and Chik viruses (63.6%, 72.3% and 48.5% respectively). Although regional viriations of the results were recorded but a general rule was postulated: the positive rate and the mean antibody titer increased progressively with age in all of the 4 study areas. The other sero—surveys conducted in 1981—1989 were summarized in Table 4. From the above results, it was noted that: depending on areas, the primary DEN infection rate in 14 localities oscillated between 6.4 to 23%. The secondary infection increased by life time and most of the adults were immunized with DEN viruses. The human susceptibility varied upon localities but the highest rate was found in children groups.

-Longitudinal sero-surveys: see Table 5. Latent activities of DEN viruses were expressed by the seroconversion by year in children. In 1986, serum samples were collected from children at creches and kindergartens at Ben Luc, Tan An, My Tho, Long Xuyen, Rach Gia. After 1 year, the second serum samples were taken from children in the same cohorts. Following up the sero-conversion in children from 1986 to the 1987 pre-epidemic stage, it was noted that: the primary infection rate increased from 10 to 30% in the pre-epidemic stage of the 1987 DHF outbreak, the susceptibility rate decreased from 80 to 40%. These results explained that the silent transmission of DEN virus had been occurring prior to the widespreading of DHF epidemic and these remarks could be considered as the risk factor for

Table 4. The human immune status by ages and years in some provinces.

T 192	No of	Primary infection						Second. infection	Suscep
Localities	sera	DEN -1	DEN -2	DEN -3	DEN -4	%	-	≥2×DEN %	tible %
1981 D.10—HCM city 5—14y	445	39	34	1	18	20.60	•	38.40	40.80
1983 SDa-DMC Tay Ninh	165						70.30		29.70
Cay Gao – V. An Dong Nai	164						50.60		49.40
1984 SDa-DMC	214						82.20		17.80
1985 Long Xuyen	315	4	4	7	16	9.84		69.52	20.63
1986					-	<del></del>			
Vam Co	188	5	3	6	4	9.57		82.97	7.44
My Tho	234	9		12	5	11.11		44.87	44.01
Can Tho 1-4y	154	1	3		6	6.40		7.10	86.30
Rach Gia 1-4y	290	1	4	29	1	12.00		16.50	71.30
1987 Hiep Thuong DQ-DNai	223						39.40		60.60
Tan Phu-DNai	111	4	16	2		19.82		41.44	38.73
1988					***************************************				
Ca Mau	276	23	5	6	24	21.01		55.43	23.55
1989							_		
Bao Loc	502	24	21	16	54	22.90		69.72	7.37
Ben Tre	232	39		6		19.40		78.45	2.16

y: years old.

Long Xuyen

Rach Gia

Localities	No of sera		Prim	ary infect	Second. infection	Susceptible		
		DEN -1	DEN -2	DEN -3	DEN -4	%	≥2×DEN %	%
1986								
B.Luc,T.An	325	3	20	8	3	10.4	23.3	66.1
My Tho	338	8	6	8	13	10.3	18.3	71.3
Long Xuyen	241	15	7	5	6	13.6	5.8	80.4
Rach Gia	290	1	4	29	1	12.0	16.5	71.3
1987								
B.Luc,T.An	281	61	22	3		30.6	25.2	44.1
My Tho	338	2	13	89	1	31.0	3.8	65.0

13

10

5

23.5

29.8

37.6

28.2

38.9

41.9

**Table 5.** Primary, secondary infection and the DEN endemic strains circulating in children of 1-4 year-old-group at 4 localities during 1986-1987

a community, a precursory sign to predict an epidemic.

2

32

16

221

124

- The inapparent infection and the circulation of DEN endemic stains: As mentioned above, second serum samples were taken from children who had had negative results or monotypic responses, if the second serum become positive or having non-monotypic response, all these children are considered to be infected inapparently by the DEN endemic strains.
  - -Studies in Ho Chi Minh City, Vung Tau, My Tho in 1979 (Do et al., 1983).
  - \*In Ho Chi Minh City, the result of examination of 96 paired sera has shown that the DEN-2 was in circulation and the rate of JE inapparent infection was 7/96.
  - \*At Vung Tau, among 49 observed children, recent DEN infection was not detected, but JE and Chik inapparent infections have occurred.
  - \*At My Tho with 65 paired sera, the circulations of DEN-3 and DEN-4 viruses were demonstrated and JE inapparent infection rate was 5/65.
- -Inapparent infection and DEN endemic sero-type at Long Xuyen, 1986: see Table 6. In 1986, at Long Xuyen there were 27/33 children (81.8%) who were still kept negative status as in 1985, 6/33 children have had sero-conversions: 4 children contracted primary infections and 2 previously infected primarily have contracted secondary infections.
- —The inapparent infection and the DEN endemic strains circulating at Long An, My Tho, Long Xuyen and Rach Gia, 1987: see Table 7. In 1986, 335 children without DEN antibodies and 37 with monotypic responses were studied. At the end of 1987 the sero—conversion of their sera was still negative, the rate was from 42 to 73%; contracted primary infection: from 25 to 34%; contracted secondary infection: from 1.3 to 27%. The endemic strains circulating were different depending on areas, such as DEN−2 in Ben Luc, Long Xuyen, Rach Gia; DEN−1 in Tan An and DEN−3 in My Tho.
- -The inapparent infection and the DEN endemic strains circulating at Ca Mau -Minh Hai, 1988: see Table 8. Among 70 children without DEN antibodies or primarily in-

fected, after the 1988 epidemic stage their results were changed as follows: negative become primarily infected: 9/70; negative become secondarily infected: 14/70; primarily infected become secondarily infected: 15/70. In general, there was 54.2% of children who have had sero—conversion.

**Table 6.** The sero-conversion in 33 children who had had negative results or monotypic responses at Long Xuyen, 1986

1	1985		1986							
1			%	Prim.inf.	%	Sec.inf.	%			
Negative:	31 children	27	87.1	4 DEN-1	12.9	0	0			
Prim.inf.:	2 children					2				

**Table 7.** The sero—conversion in healthy children having first negative sera or primary infected in 1986 and whose second sera were retested in 1987

Localities	1006		1987							
Locanties	1986		(-)	%		Prim.inf.	%	Sec.inf.	%	
	Negative: children	126	55	43.7		DEN-1:28				
Ben Luc Tan An					43	DEN-2:12 DEN-3: 3	34.1	28	22.2	
Ian An	Prim.inf.: children	13			6	DEN-3. 3	6/13	7	7/13	
	Negative: children	76	56	73.7	-	DEN-1: 1				
My Tho					19	DEN-2: 2 DEN-3:16	25.0	1	1.3	
	Prim.inf.: children	1			1					
	Negative: children	63	28	44.4		DEN-1: 1				
Long Xuyen					18	DEN-2:12 DEN-3: 5	28.6	17	27.0	
	Prim.inf.: children	12			7		7/12	5	5/12	
	Negative: children	70	30	42.9		DEN-1: 4			-	
Rach Gia					21	DEN-2:10 DEN-3: 5 DEN-4: 2	30.0	19	27.1	
	Prim.inf.: children	11			5		5/11	6	6/11	

	cu at the chu o.	1 1300						
Δ*	1/1000				Dec./198	8		
Aprı	1/1988	(-)	%		Prim.inf.	%	Sec.inf.	%
Negative:	39 children	16	41.0		DEN-1: 1			
				9	DEN-3: 6	23.0	14	35.8
					DEN-4: 2			
Prim.inf.:	31 children			16		51.6	15	48.3

**Table 8.** The second sera of 70 children who have had first negative results or primary infected were tested at the end of 1988

# A proposed strategy for the control of DHF in South Vietnam

The current option available for prevention and control of DHF epidemic is to eradicate the principal vector *Aedes aegypti*. Unfortunately the normal course that we have been applying up to now is to wait until epidemic occurs and thereafter implement control measures. This approach generally had little effect on the course of an epidemic because the DHF iceberg to this period is too big in the meaning of morbidity, lethality and inapparent cases. In the public health, the social and economic sectors impact of DHF was also a great disaster. Therefore in the whole DHF aspect, the old attitude must be changed and a new strategy designed to prevent actual epidemic transmission is a high priority. The basic problem is that the prevention and control of DHF must be started immediately in the beginning of the silent transmission period prior to the epidemic stage by using the effective active surveillance which based on serological and clinical surveillance. These surveillances aim to provide early and precise information to the public health service for starting the active control. If a DEN transmission can be detected early enough, an effective emergency mosquito control can be implemented immediately at that moment, and major DHF epidemic might be prevented (Gubler, 1987).

In South Vietnam the basic proofs for implementing the active DHF prevention and control are based on, in the first trimester of every year.

- (1)—DHF patients have been already detected.
- (2)—Inapparent and confirmed DHF cases have been serologically diagnosed.
- (3)—DEN viruses have been isolated in this period.

Only (1) and (2) are sufficient proofs to confirme that the DEN transmission has started. Therefore it must be the time of choice to implement the active DHF control programme with the objective to transfer the mosquito control programme from the Government to every person living in the community. It must be emphasized and addressed to the individual responsibility of all persons and they must be made to understand that DHF exists only because they allow mosquitoes to breed in and around their home. Persons living in communities must be educated to accept responsibility for their own health destiny and for their own family too. For carrying out the mosquito eradication project successfully, the highest responsibility belong to the Authorities at all levels, who will direct and motivate all the people to participate in this programme.

In conclusion, in front of the constant increase of DEN prevalence in Vietnam, an

effective national line for DHF prevention and control should be achieved. We hope that an active surveillance which combine with both emergency and long—term community—based vector control programme will be performed in a very near future.

#### CONCLUSION

- 1) In South Vietnam, from 1963 to 1992, DHF has occurred every year. The DHF prevalence has increased annually and major epidemic occurring every 3 to 4 years. In the largest 1987 outbreak, the province which had a highest morbidity was Vung Tau: 1,614/100,000 population and the province with highest lethality was Minh Hai: 12/100,000 population.
- 2) DHF cases were recorded all year round and epidemics have usually occurred from June to November with peak transmission in July—August or September which have closely related with the rainy season—the breeding period of mosquito vector.
- 3) DHF patients were mostly children, the most affected age was 5-6 year-old-group and the sex was not a risk factor in DHF.
- 4) Numerous DEN viruses have been isolated from DHF patients' blood as well as from mosquitoes. The dominant sero-type of the 1987 outbreak was DEN-2. In 1990 the DEN-1 appeared and slowly increased, since 1992 DEN-2 was reintroduced and slowly grown-up.
- 5) Genomic structure of DEN-2/Vietnam/87 was somewhat different in comparison with many topotypes in the world.
- 6) From 1978 to 1982, six Chik virus strains were isolated from patients diagnosed as DHF.
- 7) Serological investigations of healthy persons in 12/17 southern provinces have demonstrated that there were relatively wide circulations of DEN, JE and Chik viruses. The sero-type of DEN endemic strains were found different in some studied areas.
- 8) The inapparent DEN infections have been detected in all studied areas.
- 9) There were augmentation of the primary infection rate and decrease of the susceptibility rate in children prior to the DHF epidemic.
- 10) Seven strains of JE virus were isolated from DF patients' blood and this is possibly the concurrent infection between the two epidemic processes.

#### ACKNOWLEDGMENTS

We thank Dr. D.J. Gubler in CDC Colorado and Dr. J. L. Ladinsky in the University of Wisconsin — Madison USA for the advices and for supplying us many biological products and cell cultures during this study.

#### REFERENCES

- 1) Anonymous (1980): Guide for diagnosis, treatment and control of DHF. 2nd ed. WHO, Geneva.
- 2) Clarke, D. H. & Casals, J. (1958): Techniques for haemagglutination and haemagglutination—inhibition with arthropod—borne viruses. Am. J. Trop. Med. Hyg., 7: 561-573.
- 3) Deubel, V., Nogueira, R. M., Drouet, M. T., Zeller, H., Reynes, J. M. & Do Quang Ha (1993): Direct sequencing of genomic cDNA fragments amplified by the polymerase chain reaction for molecular epidemiology of Dengue 2 viruses. Arch. Virol., 129: 197–210.
- 4) Do Quang Ha & Duong Bich Thuy (1982): Epidemiological and virological study on DHF in South Vietnam during 1978-1980. Part 2: Serological study. J. Med., 110: 10-26 (in Vietnamese).
- 5) Do Quang Ha, Duong Bich Thuy, Truong Bich Thuy & Tran Ngoc Mai (1983): DHF in the Southern part of Vietnam during 1975-1982. Dengue Newsletter WHO, 9: 61-73.
- 6) Do Quang Ha, Tran Tien, Truong Bich Thuy, Truong Uyen Ninh & Nguyen Huu Tam (1985): Epidemiology of DHF in Vietnam during 1975—1983. Dengue Newsletter WHO, 11: 89—99.
- 7) Do Quang Ha, Nguyen Kim Tien, Dinh Tuyet Hoa, Vu Thi Que Huong, Nguyen Trong Lan, Vo Dinh Tham & Nguyen Thi Hong (1989): Epidemic DHF in South Vietnam, 1987. Dengue Newslettre, WHO, 14: 46-57.
- 8) Do Quang Ha, Vu Thi Que Huong, Truong Thi Huynh Hoa, Dinh Quoc Thong, Tran Hue Tam & Tran Ngoc Duong (1990): Some data on DHF/DSS epidemic in the Socialist Republic of Vietnam, 1988. Dengue Newsletter WHO, 15: 41-44.
- 9) Do Quang Ha, Vu Thi Que Huong, Dinh Quoc Thong, Tran Hue Tam & Nguyen Trong Lan (1991): Some data on DHF/DSS epidemic in the Socialist Republic of Vietnam, 1989. Dengue Newsletter WHO. 16: 40-44.
- 10) Do Quang Ha, Vu Thi Que Huong, Huynh Thi Kim Loan & Dinh Quoc Thong (1992): DHF/DSS epidemic in South Vietnam during 1975—1990 and its control strategy. J. Trop. Infect. Diseases, Publ. Health Assoc. HCM City, 4: 27-32 (in Vietnamese).
- 11) Do Quang Ha, Vu Thi Que Huong, Huynh Thi Kim Loan & Dinh Quoc Thong (1993): DHF/DSS epidemic in South Vietnam during 1975—1990 and its control strategy. J. Trop. Infect. Diseases, Publ. Health Assoc. HCM City, 12: 42—49 (in Vietnamese).
- 12) Doan Xuan Muou, Nguyen Hiep Nhat Lang, Do Quang Ha, Nguyen Thi Thanh, Nguyen Kim Viet, Nguyen Huy Sung & Nguyen Van Hieu (1970): Etiology of the 1969 DHF epidemic in North Vietnam. J. Med., 3: 16-23 (in Vietnamese).
- 13) Doan Xuan Muou, Nguyen Hiep Nhat Lang, Do Quang Ha, Nguyen Thi Thanh & Nguyen Kim Viet (1971): Serological studies of the 1969 DHF in North Vietnam. Proc. of the Sci. Research NIHE, 1: 70-78 (in Vietnamese).
- 14) Gubler, D. J. (1986): Application of serotype specific monoclonal antibodies for identification of dengue viruses. Arbovirus Cultivation in Arthropod Cells in Culture. Yunker, C., CRC Press, Boca Raton, 2: 3-14.
- 15) Gubler, D. J. (1987): Current research on dengue. Current Topic in Vector Research. Ed. Kerry F. Harris, Springer-Verlag, New York, 3: 37-56.
- 16) Henchal, E. A., McCown, J. M., Seguin, M. C., Gentry, M. K. & Brandt, W. E. (1983): Rapid identification of dengue virus isolates by using monoclonal antibodies in an indirect immunofluorescence assay. Am. J. Trop. Med. Hyg., 32: 164-169.
- 17) Igarashi, A. (1978): Isolation of Singh's Aedes albopictus cell clone sensitive to dengue and

- chikungunya viruses. J. Gen. Virol., 40: 531-544.
- 18) Kuno, G., Gubler, D. J., Velez, M. & Oliver, A. (1985): Comparative sensitivity of three mosquito cell lines for isolation of dengue viruses. Bull. WHO, 63: 279-286.
- 19) Kuno, G., Gomez, I. & Gubler, D. J. (1987): Detecting artificial anti-dengue IgM immune complexes using an enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg., 36: 153-159.
- 20) Nguyen Thi Kim Thoa, Pham Hung Cuong & Nguyen Van Dong (1971): Quelques données sur la fièvre hémorragique au Sud Vietnam de 1963 à 1971. Rap. ann. sur le fonct. tech. Inst. du Pasteur-Vietnam: 113-154.
- 21) Rico-Hesse, R. (1990): Molecular evolution of dengue viruses type 1 and 2 in nature. Virology, 174: 479-493.
- 22) Rosen, L. & Gubler, D. J. (1974): The use of mosquitoes to detect and propagate dengue viruses. Am. J. Trop. Med. Hyg., 23: 1153-1160.
- 23) Tran thi Tram (1991): Clinical manifestations of virological confirmed DHF/DSS patients in 1990. Sym. DHF/DSS in South Vietnam. Center of Trop. Diseases HCM City: 14-15 March.
- 24) Umenai, T. & Suzuki, H. (1990): Review on DF/DHF situation in the Western Pacific Region in 1988. Dengue Newsletter WHO, 15: 21-24.