

## **Dengue epidemic in the state of Rio de Janeiro, Brazil, 1990–1: co-circulation of dengue 1 and dengue 2 serotypes**

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### SUMMARY

During 1990 and 1991, dengue fever was detected in the State of Rio de Janeiro, Brazil. It occurred in two epidemic waves; one, from January to August 1990, caused predominantly by dengue virus type 1 (DEN-1) the other from October 1990 to May 1991 caused by type 2 virus (DEN-2). Dengue was confirmed by virus isolation and/or IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) in 2109/5964 (35·4%) of the cases. DEN-2 virus was isolated from 180 patients. HAI tests indicated that of these previous infection with DEN-1 had occurred in 130 (72%). The epidemic was classified as dengue fever, but severe and even fatal cases occurred in association with secondary infection.

### INTRODUCTION

Dengue virus was reintroduced in the State of Rio de Janeiro, Brazil during 1986 after more than 50 years, and the infection behaved as virgin soil epidemic [1–3]. The epidemic caused by dengue virus type 1 (DEN-1) involved populations in the State of Rio de Janeiro and along the eastern coast of Brazil. During 1986–7 about 60% of notified dengue cases in the Americas occurred in Brazil [4].

DEN-1 virus was the only serotype detected in the State of Rio de Janeiro until April 1990 when DEN-2 virus was isolated from a classical case of dengue fever in Niterói [5]. Active epidemiological surveillance was the key to early identification of the new serotype. DEN-2 virus was also isolated in the States of Tocantins (P. Vasconcelos, personal communication) and Alagoas (I. Rocco, personal communication) showing the rapid spread of the virus.

Sequential infection with different serotypes of dengue virus is an accepted risk factor for dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [6, 7].

In this report we present virological, serological, epidemiological, and clinical data obtained from suspected cases of dengue during the 1990–1 epidemic in the State of Rio de Janeiro.

## MATERIALS AND METHODS

*Case definition and reporting of the cases*

Dengue patients include in this study presented with high fever, headache, retrobulbar pain, myalgia, anorexia, arthralgia, rash, weakness, prostration, and haemorrhagic manifestations particularly petechiae, epistaxis and tourniquet-positive test. Dengue infection was confirmed in patients showing signs and symptoms by virus isolation and/or serology. Cases were notified by health care centres to the Rio de Janeiro Secretary of Health. Some cases were reported directly by clinicians. Serum samples were sent to the Department of Virology accompanied by clinical and epidemiological data.

*Virus isolation*

This was attempted by inoculation into clone C6/36 of *Aedes albopictus* cells [8]. Monolayers were prepared in Leibovitz (L-15) medium supplemented with 1% non-essential aminoacids, 10% tryptose phosphate and 10% foetal bovine serum. Acute serum samples diluted 1:10 in L-15, were inoculated and incubated at 28 °C for 7–10 days. Virus isolates were typed by indirect fluorescent antibody test (IFAT) using serotype-specific monoclonal antibodies [9, 10].

*Serological methods and antigens*

*IgM capture enzyme-linked immunosorbent assay (MAC-ELISA)*. MAC-ELISA was performed for routine serodiagnosis using serotype-specific antigens from DEN-1 and DEN-2 as described previously [11]. Briefly, microtitre plates (Immulon II, Dynatech Labs., Alexandria, VA) were sensitized with affinity-purified goat anti-human IgM antibody (Kirkegaard and Perry, Gaithersburg, MD) and blocked with 4% bovine serum albumine in phosphate buffered saline (PBS) pH 7.4. Normal human serum extracted with acetone was used as diluent. Serum was tested at 1/10, using a mixture of dengue virus antigens DEN-1 and DEN-2 (32 haemagglutinating units HAU for each virus) and conjugate diluted 1/2500.

Anti-flavivirus monoclonal antibody 6B6C-1 conjugated with horseradish peroxidase (Jackson Immuno Research Laboratories, Pennsylvania) and ABTS substrate (Kirkegaard & Perry Labs.) were used. The colour reaction was read 30 min after substrate addition on a Microreader (Organon) at 405 nm.

*Haemagglutination-inhibition (HAI) test*

HAI test were carried out according to Clarke and Casals [12] in 180 patients' sera previously known to be positive for DEN-2 isolation. Four–eight haemagglutinin units (HAU) of DEN-1 and DEN-2 antigens were used. Non-specific inhibitors and natural haemagglutinin were removed from all sera by absorption with kaolin (Sigma) and goose erythrocytes, respectively.

Classification of primary or secondary infection (sequential infection with different dengue serotypes) was based on the following criteria. All patients with no HAI antibodies (< 10) in acute sera obtained before the fourth day of illness or convalescent serum sample with HAI titre < 1280 were classified as primary infections. Infections were considered secondary in patients with a convalescent

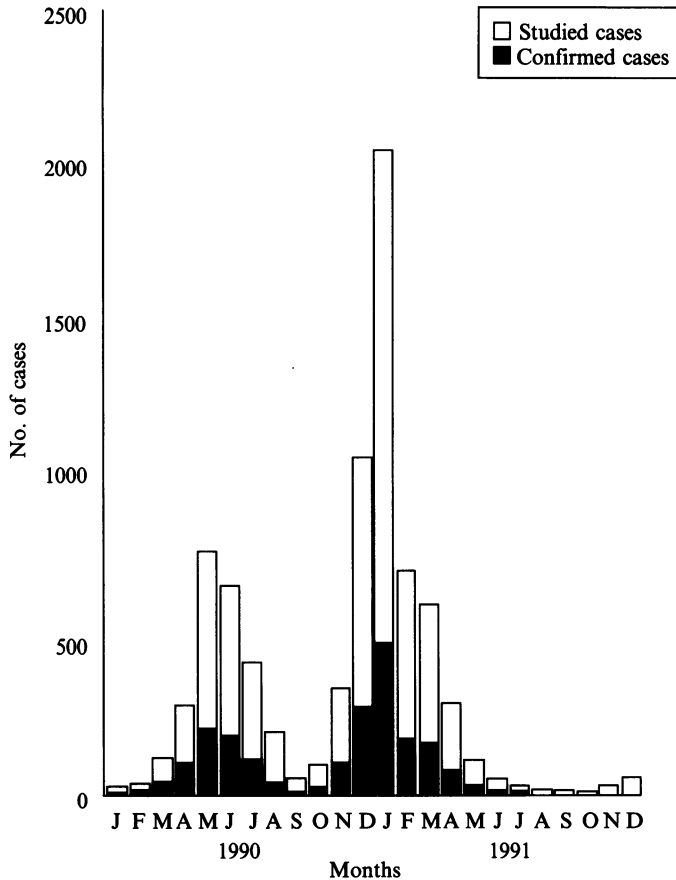


Fig. 1. Monthly distribution of dengue cases in the State of Rio de Janeiro, 1990-1.

HAI antibody titre of 1280 or greater and HAI antibody titres of 10 or greater in the acute serum [13].

#### *Antigens*

DEN-1 (Mochizuki strain) and DEN-2 (New Guinea) antigens used in serological methods were prepared from infected suckling mouse brains by the sucrose acetone extraction method [12].

## RESULTS

#### *Virological and serological results*

From January 1990 until December 1991 samples from 5964 suspected dengue cases in the State of Rio de Janeiro were submitted for laboratory tests. Of the 2474 sera tested DEN-1 virus was isolated from 225 samples and DEN-2 from 188 samples. The overall virus isolation rate was 413 (16.7%). Dengue was confirmed by virus isolation and/or MAC-ELISA in 2109/5964 (35.4%) patients. Of the patients from whom DEN-2 was isolated, 130/180 (72.2%) were classified as having secondary infection and 50/180 (27.8%) as primary infection by HAI test. In paired sera obtained from 42 cases confirmed by DEN-2 isolation, 11 showed

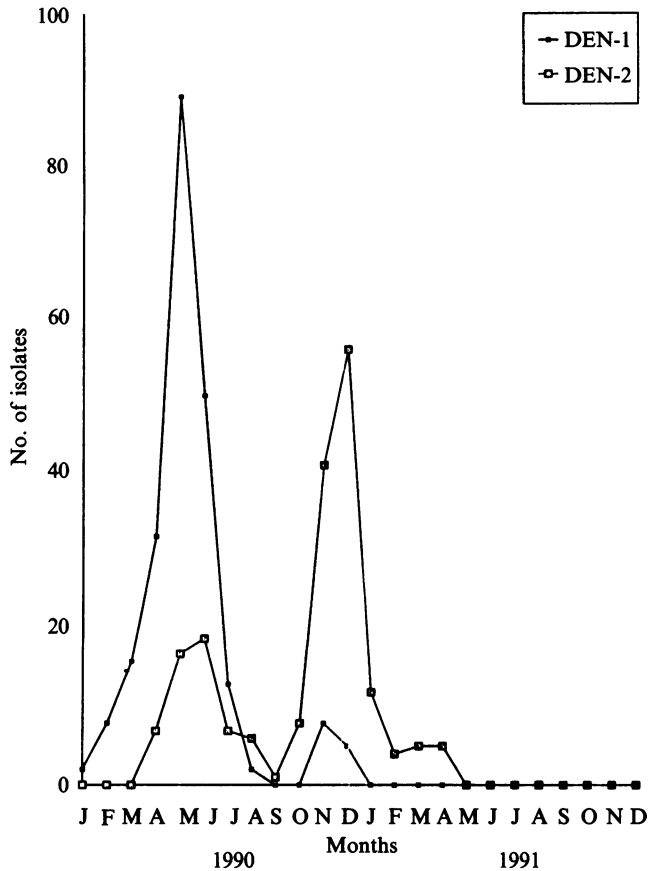


Fig. 2. DEN-1 and DEN-2 virus isolation from patients in the State of Rio de Janeiro, 1990-1.

primary infections and IgM positive responses could be detected in all of these. Among patients that showed secondary infection by HAI test, IgM antibodies were detected in 29/31 (93.5%).

#### *Epidemiological findings*

As shown in Figure 1 dengue increased in April 1990, when DEN-2 was primarily isolated, and declined in September and October. In 1991 a new peak occurred in the summer months. DEN-1 was isolated throughout 1990, although a clear predominance of DEN-2 virus was observed from August to December 1990. DEN-2 was the only serotype isolated in 1991 (Fig. 2). During 1990-1, 242 (58.6%) of the isolates of DEN-1 and DEN-2 virus came from Rio de Janeiro municipality (Table 1).

#### *Clinical aspects*

Clinical data were analysed on 390 confirmed dengue cases seen at Evandro Chagas Hospital, Rio de Janeiro, from April to July 1990, and from December 1990 to April 1991, when DEN-1 and DEN-2 were the predominant serotypes respectively. Haemorrhagic manifestations, hospitalizations and levels of platelets

Table 1. *Distribution of dengue cases by municipalities of the State of Rio de Janeiro, 1990-1*

Municipalities	1990		1991
	DEN-1	DEN-2	DEN-2
Rio de Janeiro	155	75	12
Niterói	6	41	10
Duque de Caxias	3	2	1
São Gonçalo	7	9	—
Paracambi	7	7	—
São João de Meriti	10	22	3
Nova Iguaçu	13	1	—
Nilópolis	10	4	—
Campos	3	—	—
Itaboraí	2	—	—
Bom Jesus de Itabapoana	8	—	—
Bom Jardim	1	—	—
Araruama	—	1	—
Total	225	162	26

Table 2. *Clinical and laboratory features of 390 confirmed dengue cases diagnosed in successive seasons. Evandro Chagas Hospital, Rio de Janeiro, 1990-1*

	Haemorrhagic† manifestations	Hospitalization§	Thrombocytopenia   (platelets $\leq 10^5/\text{mm}^3$ )
May 1990-July 1990*	36/126 (28.6%)	1/126 (0.8%)	3/79 (3.8%)
August 1990-April 1991†	93/264 (35.2%)	24/264 (9.1%)	49/254 (19.3%)

\* DEN 1-predominant.

† DEN-2 predominant.

‡  $\chi^2 = 1.71$ ,  $P = 0.191$ .§  $\chi^2 = 9.79$ ,  $P = 0.002$ .||  $\chi^2 = 10.98$ ,  $P = 0.001$ .

from these patients are shown in Table 2. Petechiae, epistaxis and gum bleeding were the haemorrhages most commonly observed. A significantly greater proportion of patients with thrombocytopenia and requiring hospitalizations were seen in DEN-2 predominant period. Concomitant haemorrhagic manifestations, thrombocytopenia, and a rise of hematocrit  $\geq 20\%$  in comparison with convalescence data, showing increased vascular permeability, was seen in six patients. These can be classified as cases of dengue haemorrhagic fever (DHF) grade II as defined by WHO [6].

#### DISCUSSION

Following the 1986-7 DEN-1 epidemic in the State of Rio de Janeiro, active epidemiological surveillance was established. The 1990-1 epidemic occurred after 2 years of low dengue activity and reached a total of 97 825 reported cases [14]. The first DEN-2 virus isolation occurred exactly 4 years after the detection of DEN-1 [1, 5] and probably resulted from a similar route of entry into the country during the summer time.

After the first DEN-2 virus isolation from Niterói, in April 1990 several strains were isolated from different municipalities showing rapid virus dissemination. Like the 1986–7 epidemic [3], the municipality of Rio de Janeiro showed the greatest number of confirmed cases demonstrating that vector spread and environmental conditions still favour epidemic transmission in that area despite the efforts to control the vector. Density of *Ae. aegypti* house indices higher than 5%, as defined by WHO [6] were detected in 15 different areas in Rio de Janeiro municipality. In five of them densities were > 9% before vector control measures were started during 1991 (M. Lima, personal communication).

The number of dengue reported cases decreased after April 1991 when the last virus isolation occurred. Dengue virus has not been isolated since. The increase of DEN-2 from August 1990 and the fact that only this serotype was isolated in 1991 can be explained by the immune status of the population against DEN-1 and the susceptibility to the new serotype.

The lower virus isolation rate (16.7%) during the 1990–1 epidemic compared to 41.2% during the DEN-1 epidemic in 1986–7 [3] may be explained by the greater number of secondary infections in the former epidemic. The high levels of antibody detected in acute phase of disease due to secondary infection would make virus isolation less feasible [15]. Nevertheless, DEN-2 was isolated from 180 patients of which 150 were confirmed as secondary infections. The early collection of blood samples in these infections and the high sensitivity of the cell line probably explain these findings.

The IgM response in secondary dengue is said to be suppressed, often to undetectable levels. However Pang, using the haemadsorption immunosorbent technique, showed that 36/41 sera from patients with secondary dengue had significant IgM antibodies titres [16]. In our experience, 93.5% IgM positivity (MAC-ELISA) was observed in 31 paired sera from patients with DEN-2 isolation, classified as secondary infection. A high percentage of IgM response in paired sera was also found by Chungue and colleagues in French Polynesia [17].

The increase from 16.7 to 35.4% confirmed cases, when both serology and virus isolation were used, clearly demonstrates the usefulness of MAC-ELISA even in secondary infections. We stress the value of testing paired sera in order to confirm dengue infection in cases where no virus can be isolated.

The introduction of DEN-2 into Rio de Janeiro resulted in the appearance of more severe diseases including cases of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) [18]. Clinically, the epidemic was characterized as dengue fever, although the Ministry of Health reported 435 patients with DHF and 3 deaths from November 1990 to November 1991 [19].

The clinical manifestations of 390 confirmed cases were different in the two periods of the epidemic. Although there was no significant difference in the rate of haemorrhagic manifestations observed during whole epidemic, the high proportion of patients hospitalized and presenting with platelet counts of  $\leq 10^5/\text{mm}^3$  from August 1990–April 1991 showed that the disease was more severe in the period of greater circulation of DEN-2.

Two studies carried out in different hospitals, one in Rio de Janeiro [20] and another in Niterói city (Zagne and colleagues, unpublished) showed that DHF occurred most frequently as DHF grade I and II [6].

From the epidemiological point of view the dengue epidemic in the State of Rio de Janeiro did not reproduce the severe features of the Cuban experience of DHF epidemic in 1981, although the same sequence of serotypes occurred. In Cuba 344203 dengue cases were reported, 10312 were classified as WHO grades II-IV and 158 died, after sequential epidemics of DEN-1 in 1977-8 and DEN-2 about 4 years later [21]. Other factors such as virulence of strains are certainly involved. The increase in the incidence of dengue, the frequency of epidemic episodes and the number of dengue serotypes circulating in the Americas have been pointed out as factors responsible for the emergence and spread of DHF/DSS [4].

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