

Retrospective Study on Dengue in Fortaleza, State of Ceará, Brazil

RV da Cunha, MP Miagostovich^{*/+}, Z Petrola^{**}, ESM de Araújo^{*},
D Cortez^{***}, V Pombo^{**}, RV de Souza^{****}, RMR Nogueira^{*},
HG Schatzmayr^{*}

Departamento de Clínica Médica, Universidade Federal de Mato Grosso do Sul, Curso de Pós-Graduação (Doutorado) em Medicina Tropical, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil *Laboratório de Flavivírus, Departamento de Virologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil **Laboratório Emílio Ribas, Fortaleza, CE, Brasil ***Departamento de Epidemiologia, Secretaria Estadual de Saúde, Fortaleza, CE, Brasil ****Hospital Evandro Chagas, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

A retrospective serologic study was carried out in Fortaleza, State of Ceará, Brazil, in order to detect the dengue virus activity before recognizing the epidemic of 1994. Mac-Elisa was performed by using a mixture of specific DEN-1 and DEN-2 antigens on serum samples from the Emilio Ribas Laboratory collection. Samples were obtained from 1,224 patients with exanthematic febrile disease and negative serological results for rubella. All specimens were taken during November 1993 to May 1994. The results confirmed dengue infections in Fortaleza by November 1993, approximately six months before the beginning of the epidemic, proving how misleading diagnosis of dengue infection are still troublesome, in spite of the strong dengue activity in Ceará. The authors stress the urgent necessity to implement the active surveillance system in order to prevent another extensive dengue fever epidemics in the state. Epidemiological background of the dengue activity in the State of Ceará is also described.

Key words: dengue - retrospective serologic study - Ceará - Brazil

The re-introduction of *Aedes aegypti* into Brazil in 1967 and its rapid spread through the country presented the dengue infection as a national public health problem, with more than 500,000 dengue reported cases during the last decade (Figueiredo 1996).

The Northeast region has been suffering successive dengue epidemics in that period, and the State of Ceará is the second most infected state of the country according to reported dengue cases. From 1986 to 1996, the state reported a total of 102,010 cases as a result of the three epidemics which occurred at intervals of two years each (Araújo et al. 1996).

In this report we describe the epidemiological background of dengue activity in Ceará, as well as the results of a retrospective serologic investiga-

tion carried out in Fortaleza, the capital of the state. The objective of the study was to detect dengue virus activity before the initial recognizing of the epidemic of 1994.

Epidemiological background - Re-introduced in Ceará during the mid-80s, in the cities of Aquiraz, Beberibe and Fortaleza (Lima 1985), *Ae. aegypti* found adequate conditions to begin its dispersion. In 1984 official reports showed that 3 out of the 44 (6.8%) municipalities of the state were infested. Then, in 1988 it increased to 70.8% and in 1992 dropped to 46% (FNS 1992).

By August 1986, DEN-1 virus caused dengue outbreak in the state, with a peak reported in April 1987. In the following year, the disease continued to spread, with new cases being reported during the last months of 1988 up to September 1989, when a new outbreak had began. During 1990-1991 the state reported a total of 22,434 cases and from October 1992 to February 1994 only 16 cases have been reported.

However, in March 1994 dengue transmission increased significantly and by April, the Central Laboratory of Public Health of the State of Ceará (LACEN-CE) confirmed dengue infection in 38% (41/108) of the tested cases by Mac-Elisa (LACEN 1994). In the following month, DEN-2 virus was

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⁺Corresponding author. Fax: + 55-21-270.6397

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isolated by the Flavivirus Laboratory, Institute Oswaldo Cruz (Souza et al. 1995). DEN-1 virus was also isolated in Ceará during 1994 showing the co-circulation of both serotypes in the state (Vasconcelos et al. 1995).

The first dengue cases of the epidemic of 1994 were reported in Fortaleza, which had environment conditions appropriate for high dengue activity. The interruption of measures of vector control during 1993 resulted in house index of *Ae. aegypti* superior to 20% in the capital (FNS 1993). The beginning of the rainy season along with temperatures around 28°C (Fig. 1) contributed for the explosive epidemic that rapidly spreaded to the northeast of the state. In that year Ceará was responsible for 84% of dengue cases in Brazil, reporting a total of 47,889 cases (Araújo et al. 1996). Fig. 2 shows the increasing of dengue reported cases in the state during the last eleven years.

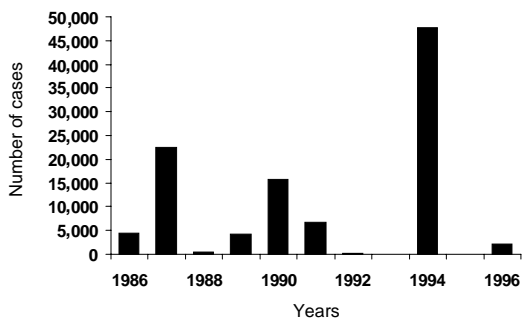
MATERIALS AND METHODS

RETROSPECTIVE SEROLOGIC STUDY

Population studied - It was 1,224 patients with exanthematic febrile disease and negative serological results for rubella.

Specimens - Serum samples obtained from the Emilio Ribas Laboratory collection, in Fortaleza. Blood samples were obtained from November 1993 to May 1994 and the sera were stored at -20°C until used for serology.

Serology - Mac-Elisa (IgM capture enzyme linked immunosorbent assay) for dengue was performed according to Kuno et al. (1987). A mixture



Source: DEEPI/SESA-CE

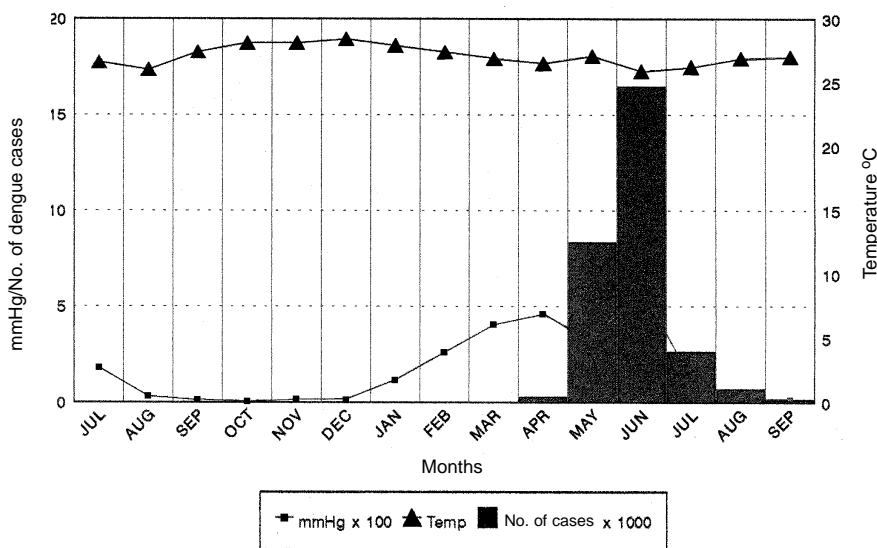
Fig. 2: reported dengue cases in the State of Ceará, 1986-1996.

of specific DEN-1 and DEN-2 antigens was used due to the co-circulation of both serotypes in the state.

Antigens - The DEN-1 (Mochizucki strain) and DEN-2 (New Guinea C) antigens were prepared from infected brains of suckling mice by using the sucrose acetone extraction method (Clarke & Casals 1958).

RESULTS

Dengue diagnosis were serologically confirmed in 24.4% (299/1,224) of studied cases by the detection of anti-dengue IgM antibodies. From 90 pairs of sera, 17 demonstrated seroconversion by Mac-Elisa. In 10 pairs only first sample of sera showed positive results showing the transitory nature of this antibodies. The remaining 63 pairs of sera showed negative results in both samples.



Source: DEEPI/SESA - CE FUNCEME

Fig. 1: environment conditions and reported dengue cases in Fortaleza, CE, 1993-1994.

Table shows the detection of anti-dengue IgM antibodies of the studied samples distributed by month.

The comparison between of the number of dengue cases confirmed by Mac-Elisa and the number of reported cases by SESA-CE in that period showed dengue activity in the state before recognizing the epidemic of 1994 (Fig. 3).

DISCUSSION

Difficulties in controlling vectors and misleading diagnosis on dengue infections seems to be the most important factors responsible for the extensive dengue epidemics in Brazil.

In spite of the endemicity of dengue viruses in the country, late diagnostic are still frequent and

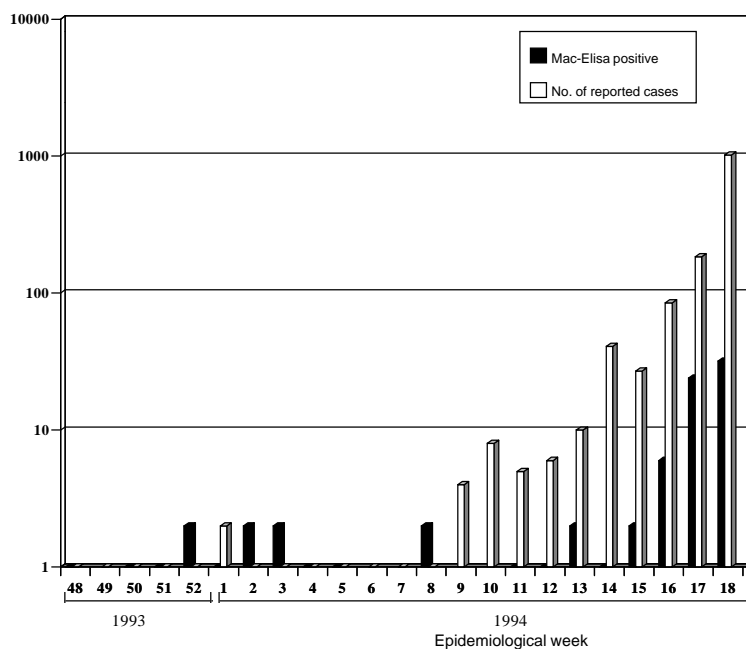
rubella seems to be the mainly disease for differential diagnosis (Nogueira et al. 1995).

In Ceará dengue fever diagnosis was misleading and the Epidemiological Health System pointed out rubella as the current infection in that time. In fact, our retrospective study confirmed serologically dengue infection in 24.4% of suspected rubella cases. Even by April 1994, when a dengue epidemic was laboratorially confirmed by LACEN-CE, 158 patients with exanthematic febrile disease were still leaded to the Emilio Ribas Laboratory in order to confirm rubella infection. Later, our study confirmed dengue infection in 20.9% (33/158) of this cases.

The detection of IgM antibodies in 24.4% (299/1,224) of the samples studied must be considered

TABLE
Distribution of 1,224 cases tested by Mac-Elisa

Year	Month	Epidemiological week	No. of positive/No. of tested (%)
1993	Nov.	44th 45th 46th 47th 48th	1/13 (7.7)
	Dec.	49th 50th 51st 52nd	4/45 (8.9)
1994	Jan.	1st 2nd 3rd 4th	6/68 (8.8)
	Feb.	5th 6th 7th 8th	3/55 (5.5)
	Mar.	9th 10th 11th 12th 13th	4/60 (6.7)
	Apr.	14th 15th 16th 17th	33/158 (20.9)
	May	18th 19th 20th 21th	248/825 (30.6)
Total			299/1224 (24.4)



Source of reported cases: DEEPI/SESA - CE

Fig. 3: reported dengue cases and Mac-Elisa positive cases by epidemiological week in Ceará, 1993-1994.

as a high rate, specially if we take into account that the result were obtained from single serum samples, in the majority of the cases. As previously described, the current use of paired sera (acute and convalescence) increases the Mac-Elisa positivity in 30% of the samples (Nogueira et al. 1993). The seroconversion observed in 17 cases strengthens the need of collecting two samples, mainly during interepidemic periods, when epidemiological surveillance should be more rigorous to reach a conclusive diagnostic. Serologic negative results for rubella and dengue infections suggests the circulation of another etiological agent responsible for this clinical features in Fortaleza.

Although we could not determine the virus serotype circulating in the last two months of 1993, these results pointed out the need to implement a virological active surveillance system in the state. An early diagnosis should have the use of vector control measures, at least six months before the initial recognizing of the epidemic. Unfortunately, samples before November 1993 were not available in the Emilio Ribas Laboratory. According to Gubler et al. (1979), when a new serotype is introduced in a susceptible community, there is a period of silent transmission until a proportion of the infected mosquitoes reaches a critical level to cause epidemics.

Our results demonstrated a sub-notification of dengue cases mainly due to the inaccurate clinical dengue diagnosis in Fortaleza. This sub-notification along with the appearance of dengue haemorrhagic fever (DHF) cases in the state (Souza et al. 1995, Vasconcelos et al. 1995), suggests that the official numbers of reported cases are underestimated, since DHF represents only the tip of iceberg of many other cases of dengue infection (Gubler 1987).

The occurrence of DHF in Ceará confirms that this form of disease is becoming progressively endemic in Brazil as in the whole American continent, following the experience observed in Asia (Halstead 1990). The increasing of the severity of the infection after the introduction of DEN-2, virus was previously described in the State of Rio de Janeiro when a similar epidemiological situation occurred during the epidemic of 1990/1991 (Nogueira et al. 1993, Zagne et al. 1994). The sequencing of DEN-1 and DEN-2 viruses isolated in Ceará in 1986 and in 1994, respectively are similar to those viruses isolated in Rio de Janeiro in the same period (Miagostovich, pers. commun.).

As observed in Rio de Janeiro during 1990/1991 (Nogueira et al. 1993, Zagne et al. 1994), the distribution by age group of DHF cases occurred almost totally in people above 15 year-old (data not showed). This pattern of distribution is exactly

inverse of the observed in Asia (Halstead 1990), demanding new studies to detect other factors in pathogenesis of DHF besides sequential infection.

The emergence of DHF and the continuity of reported dengue cases in the state and in all over the country reinforced the importance of the laboratorial methods to a definitive characterization of dengue infection. The implementation in Brazil of good quality kits could be an alternative to improve fast dengue diagnosis. In this case, private clinical laboratories would join the Public Health Laboratory Network (Schatzmayr et al. 1996), specially during interepidemic periods, when the public sector very often do not take care of dengue activity. Virological and serological surveillance along with measures adopted by the Program of Eradication of *Ae. aegypti* are the most important tools to reach dengue infection control in the country.

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