Rev. Inst. Med. trop. São Paulo
35 (2): 149-154, março-abril, 1993

DENGUE EPIDEMIC IN THE STATE OF RIO DE JANEIRO, BRAZIL: VIROLOGICAL AND EPIDEMIOLOGICAL ASPECTS.

Marize P. MIAGOSTOVICH (1), Rita M.R. NOGUEIRA (1), Silvia M.B. CAVALCANTI (2), Keyla B.F. MARZOCHI (3) & Hermann G. SCHATZMAYR (1).

SUMMARY

Laboratorial studies were carried out on 3178 patients with signs and symptoms suggestive of dengue infection from April 1986 to December 1987 in the State of Rio de Janeiro, Brazil. The epidemic had two peaks following the first virus isolation and affected the inhabitants of 17 counties. Both sex and all age groups were affected. Dengue virus type 1 was isolated from 1039 sera and the number of confirmed cases was increased to 1874 (59%) by MAC-ELISA. Isolation rate confirmed cases reached 80% in the specimens obtained until the 4th day after the onset of disease and viraemia ranged from $10^{3.0}$ to $10^{8.5}$ $TCID_{50}$/ml.

KEY WORKS: DEN-1 Virus isolation; Brazil

INTRODUCTION

Cases of dengue fever were described in Brazil on clinical grounds[11,21] but the first dengue infections confirmed by laboratory tests occurred in 1982 in the city of Boa Vista, in Amazonian area. The outbreak was associated with serotypes 1 and 4 and 7,000 cases were estimated to have occurred[15].

An extensive epidemic of dengue type 1 (DEN-1) was recognized in the State of Rio de Janeiro in April 1986[22]. The epidemic started in county of Nova Iguaçú near the city of Rio de Janeiro (23°S/43°) and it soon spread surrounding areas and also to other states of the country[14]. Nearly 140,000 cases of dengue were reported in Brazil during 1986 - 1987, most of which in the State of Rio de Janeiro[17].

This study presents virological and epidemiological data obtained from patients with signs and symptoms suggestive of dengue infection, from April 1986 to December 1987 in the State of Rio de Janeiro.

MATERIALS AND METHODS

Specimens

Serum specimens were obtained from 3178 patients suspected of dengue infection including 1045 paired samples. Convalescent samples were obtained from 2 to 4 weeks after onset of disease, but in some cases were taken after a longer interval.

Blood samples were drawn by venipuncture and delivered to the laboratory with questionaries filled out by physicians. After centrifugation serum was removed and acute specimens were divided into two vials and stored at -70°C and -20°C. Convalescent serum specimens were stored at -20°C.

Virus isolation and identification

Virus isolation was attempted from human acute phase serum specimens independently of knowledge of their antibody content. C6/36 clone of /Aedes albopictus/ cells [7] were grown on Leibovitz medium (L - 15) supplemented with 1% non-essential aminoacids, 10% tryptose phosphate broth and 10% heated (56°C for 30 min) fetal calf serum. Sera were diluted 1/10 in tissue culture medium L - 15 and inoculated (0.05ml) onto monolayers of cells in 2.0 ml of culture medium containing 2% fetal calf sera. Tubes were kept at 28°C and observed daily for viral cytopathic effect (CPE). Monolayers which showed no CPE were initially tested for direct fluorescent assay (DFA) using FITC conjugate prepared from pooled human sera with high hemagglutination-inhibition

(1) Departamento de Virologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Caixa Postal 926. 21045-360 Rio de Janeiro, RJ, Brasil.
(2) Universidade Federal Fluminense, Rio de Janeiro, RJ, Brasil.
(3) Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil.

(HI) antibody titers. Cultures positive on DFA and all cultures with clear CPE were tested with type specific monoclonal antibodies for each serotype of dengue virus. The specific monoclonals used were 15F3 (DEN-1), 3H5 (DEN-2), 5D4 (DEN-3) and 1H10 (DEN-4)[6]. Slides were prepared according to the protocol described by GUBLER[3].

Virus titration

Forty five positive sera for dengue virus were titrated by immunofluorescence in microtiter plates as described by SCHOEPP & BEATY [23] with minor modifications. Four replicates of each serial 10-fold dilution of sera were inoculated onto C6/36 cells in microtiter plates (0.05 ml/well). The plates were kept in a humid incubador at 28°C for 10 days when part of the supernatant fluid of each well was removed and the cells were resuspended in the remaining maintenance medium. Cell suspensions were transferred to slide (Bio-Mérieux) with a Pasteur pipette and allowed to dry. The slides were fixed on cold acetone (-20°C for 20 min), air-dried, and tested by DFA. Virus titers were calculated as $TCID_{50}$/ml by the REED & MUENCH method[20].

IgM capture Elisa (MAC-ELISA)

Sera from 3103 patients were tested by an IgM capture ELISA as described by KUNO et al. [9].

RESULTS

Epidemiological findings

Dengue virus was introduced in State of Rio de Janeiro and spread in a great epidemic that involved several communities during 1986-1987. A large number of cases were confirmed in May and in June declining later on. The incidence of dengue infection was observed in following months and then another explosive epidemic occurred in the summer of 1987 (Fig. 1).

The epidemic affected 17 counties where / Aedes aegypti/ was present in high densities, but the majority of the studied cases were from Rio de Janeiro, Niterói and Nova Iguaçú (Table 1).

The median age of the male and female patients was 28 and 26 years, respectively and the number of males and females affected was not significantly different ($P>0.05$, $X^2=15.32$, Table 2).

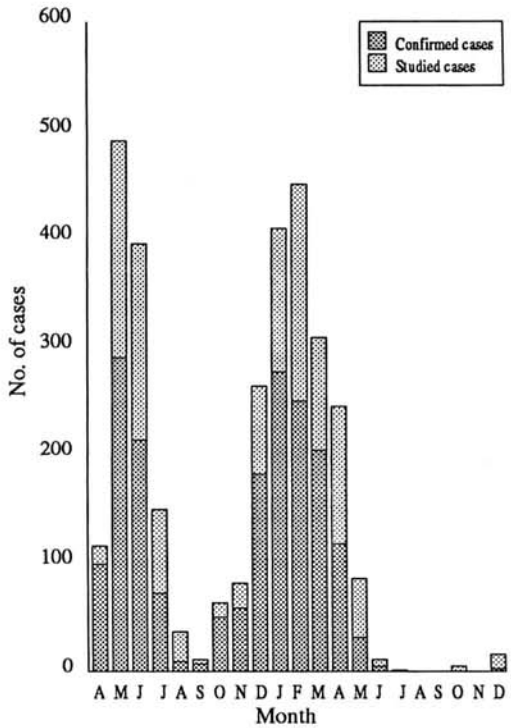

Fig.1: Monthly distribution of dengue cases, RJ, 1986-87.

Table 1
Distribution of dengue cases in different counties. RJ, 1986-87

Municipalities	Nº. of confirmed cases/ Nº. of tested
Rio de Janeiro	1057/1750
Niterói	284/417
Nova Iguaçú	171/264
Duque de Caxias	49/70
São João de Meriti	21/32
São Gonçalo	19/27
Volta Redonda	16/90
Nilópolis	6/16
Sapucaia	3/13
Petrópolis	1/10
Campos	2/8
Saquarema	4/7
Vassouras	6/7
Angra dos Reis	2/5
Magé	0/4
Friburgo	1/4
Maricá	0/2
Xerém	1/2
Teresópolis	1/1
Rio Bonito	0/1
Unknown	230/448
Total	1874/3178

Table 2
Age and sex distribution of dengue cases, RJ, 1986-87

Age \ Sex	Male	Female	Unknown	Total
0-4	13/34	10/31	-	23/65
5-9	25/62	28/48	-	53/110
10-14	41/74	40/67	-	81/141
15-19	65/107	61/102	-	126/209
20-29	217/433	258/263	-	475/796
30-39	199/350	187/333	-	386/683
40-49	134/170	130/185	-	264/355
>50	128/128	81/180	-	209/308
Unknown	119/232	137/276	1/3	257/511
Total	941/1590	932/1585	1/3	1874/3178

Virological results

Only DEN-1 virus was isolated from 1039 specimens (41.2%) of which 937 isolates produced a marked syncytial cytopathic effect (CPE) often visible around six days after inoculation (Fig. 2). Dengue antigen was detected in 102 cultures with no CPE. High rates of isolation (about 80%) could be obtained until the 4th day after onset of disease in laboratory confirmed cases (Table 3). Circulating DEN-1 virus titers ranged from $10^{3.0}$ to $10^{8.5}$ $TCID_{50}$/ml on days 1 through 8 of disease (Table 4).

The number of confirmed cases was increased to 1874 (59%) by MAC-ELISA. In 380 opportuni-

Table 3
DEN-1 isolations in confirmed cases of dengue infections in relation to onset of disease.

Day after onset of disease	Dengue type 1 isolates/Total of samples	% Virus isolation
1	30/38	78.9
2	250/278	89.9
3	216/250	86.4
4	222/266	83.5
5	104/177	58.8
6	33/104	31.7
7	13/84	15.5
8	4/29	13.8
Unknown	167/304	54.9

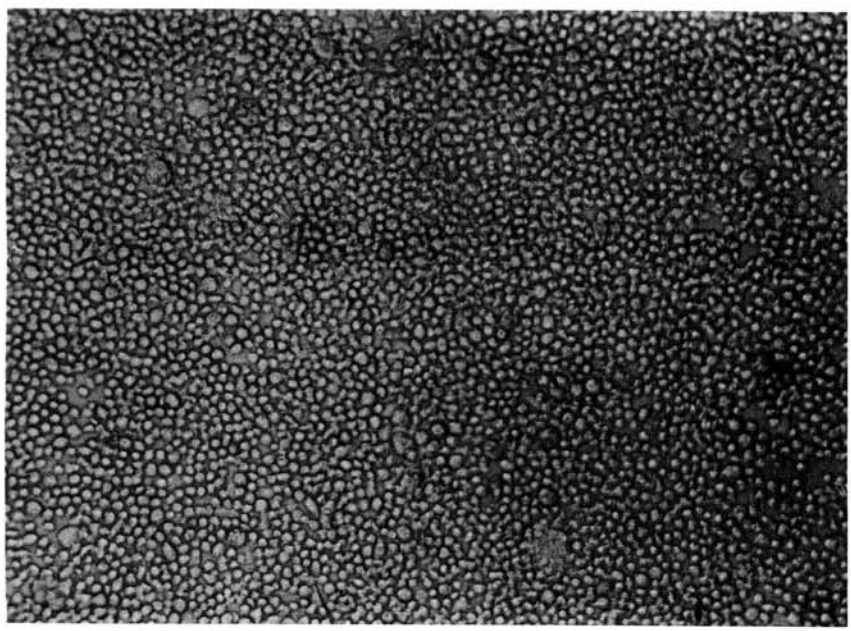

Fig. 2 A: Isolation of dengue virus in Aedes albopictus cell line clone C6/36. Cell control (100x).

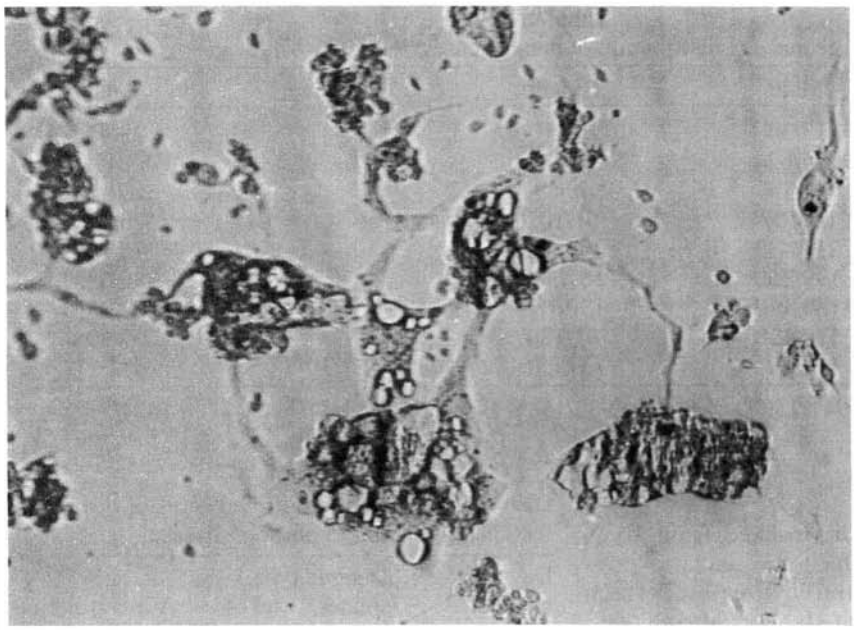

Fig. 2 B: Isolation of dengue virus in Aedes albopictus cell line clone C6/36. Infected cells showing advanced cytopathic effect (100x).

Table 4
Geometric mean virus titers in sera from patients with dengue infection by day of disease.

Day after onset of disease	nº. of sera	Virus titer range	Geometric mean ± SD*
1	7	3.6-7.8	5.4±1.7
2	7	3.9-6.0	4.7±0.7
3	8	3.1-6.6	4.2±1.3
4	5	4.3-6.8	5.3±0.9
5	6	4.1-8.5	5.9±1.6
6	5	3.6-8.1	5.5±1.6
7	5	3.8-6.1	5.2±0.8
8	2	3.0-5.3	4.4±1.6
Total	45	3.0-8.5	5.1±1.3

*Log_{10} $TCID^{50}$/ml

ties virus isolation and positive IgM response could be detected in the same patient.

DISCUSSION

The outbreak started in Nova Iguaçú, a county near Rio de Janeiro city. Nova Iguaçú has ideal conditions for dengue transmission including extensive mosquito breeding grounds and intense human crowding. The great movement of people from Nova Iguaçú to Rio de Janeiro, where high mosquito densities were also found, readily explains the spreading of dengue outbreak resulting in an extensive epidemic in the State of Rio de Janeiro. A total of 80,000 cases were reported during this period (1986 - 1987) and serological surveys suggested that over one million persons became infected in the State[17]. Since the last dengue cases reported in the State of Rio de Janeiro occurred more than 60 years ago [16], the great majority of cases were surely primary infections. A very low circulation of arboviruses before the dengue outbreak [18] and our results on age-distribution indicate that dengue was not endemic in Rio de Janeiro prior 1986 epidemic.

The epidemic involved populations not only in

the State of Rio de Janeiro but also along the eastern coast of Brazil. After the initial rapid spread in 1986-1987, dengue virus has been isolated in Rio de Janeiro in association with sporadic disease and small outbreaks. DEN-1 virus is endemic and was the only serotype detected in coastal areas of Brazil until April 1990 when DEN-2 was isolated in the States of Rio de Janeiro[13], Alagoas* and Tocantins[25] suggesting that vector spread and environmental conditions still favour epidemic transmission.

The use of virus isolation and MAC-ELISA techniques was effective in confirming infection by dengue virus in 59% of patients. The occurrence of unconfirmed cases emphasize the need of laboratory confirmation for a definitive diagnosis of dengue as described earlier[5,12].

Several continuous mosquito cell lines have been shown to be highly susceptible to dengue virus infection[7,8,19]. In our experience, C6/36 cell line was very satisfactory and provided a useful isolation system for DEN-1 virus in the State of Rio de Janeiro. Moreover, handling of this cell clone was easy and presumptive diagnosis could be reached when CPE was detected. This method was previously described as effective for virologic surveillance of dengue virus[3]. The mosquito cell clone C6/36 has previously been used for isolation of dengue viruses[10,24] with high isolation rates. The high frequency of virus isolation and the high levels of viraemia observed in some patients (> $10^{8.0}$ $TCID_{50}$/ml) are consistent with the explosiveness of the epidemic. According to GUBLER[2] high human viraemia may be associated with epidemic strains of dengue virus, while lower viraemia is associated with endemic dengue virus strains.

Patients infected with DEN-1 in Indonesia had virus titers ranging from $10^{3.8}$ to $10^{8.0}$ MID_{50}/ml. Ranges and geometric mean titers showed little change up to day 6 of the illness[4]. Our results with tissue cultures are in agreement with these findings.

Clinically, the epidemic had the characteristics of classical dengue fever, the most common symptoms being fever, mialgia, headache, joint pain, nausea/vomiting, eye pain and rash[1]. Hemorrhagic manifestations were observed over the course of the epidemic and one fatal case was confirmed by virus isolation from liver[14].

* Rocco, I., personal comunication.

RESUMO

Epidemia de dengue no Estado do Rio de Janeiro, Brasil: aspectos virológicos e epidemiológicos.

Foram realizados estudos laboratoriais em 3178 pacientes com sinais e sintomas sugestivos de infecção por dengue no Estado do Rio de Janeiro, no período de abril de 1986 a dezembro de 1987. A epidemia apresentou 2 picos e afetou residentes de 17 municípios. Ambos os sexos e todos os grupos etários foram igualmente afetados.

Dengue virus tipo 1 foi isolado de 1039 soros e a utilização do MAC-ELISA elevou para 1874 (59%) o número de casos confirmados. Nestes casos, a taxa de isolamento alcançou 80% nos espécimens obtidos até o quarto dia após o início da doença. A magnitude da viremia variou de $10^{3.0}$ a $10^{8.0}$ $TCDI_{50}$/ml.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Evandro Chagas Hospital, Oswaldo Cruz Foundation for providing blood samples and to J.Farias Filho, J. Carvalho Filho for Technical assistance and to Dr. H.G. Pereira for critical review of the manuscript.

Financial support: This work was supported by the IDRC/Canada, Grant nº. 3-p-88-214 and FNS-Ministry of Health, Brazil.

REFERENCES

1. DIETZ, V.J.; GUBLER, D.J.; RIGAU-PEREZ, J.G.; PINHEIRO, F.P & SCHATZMAYR, H.G. - Epidemic Dengue-1 in Brazil, 1986. Evaluation of a clinically based dengue surveillance system. **Amer. J. Epidem., 131:** 693-701, 1990.

2. GUBLER, D.J. - Current research on dengue. In: HARRIS K.F., ed. **Current topics in vector research.** New York, Springer-Verlag, 1987. p. 37-56.

3. GUBLER, D.J.; KUNO, G.; SATHER, G.E.; VELEZ, M. & OLIVER, A. - Use of mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. **Amer. J. trop. Med. Hyg., 33:** 158-165, 1984.

4. GUBLER, D.J.; SUHARYONO, W.; TAN, R.; ABIDIN, M. & SIE, A. - Viraemia in patients with naturally acquired dengue infection. **Bull. Wld. Hlth. Org., 89:** 623-630, 1981.

5. GUZMAN, M.G.; KOURI, G.P.; BRAVO, J.; CALUNGA, M.; SOLER, M.; VAZQUEZ, S. &

VENEREO, C.-Dengue hemorrhagic fever in Cuba. II. Serological confirmation of clinical diagnosis. **Trans. roy. Soc. trop. Med. Hyg., 78:** 235-238, 1984.

6. HENCHAL, E.A.; GENTRY, M.R.; McCOWN, J.M. & BRANDT, E. - Dengue virus-specific and flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. **Amer. J. trop. Med. Hyg., 31:** 830-836, 1982.

7. IGARASHI, A. - Isolation of a Singh's **Aedes albopictus** cell clone sensitive to dengue and chikungunya viruses. **J. gen. Virol., 40:** 531-544, 1978.

8. KUNO, G. - Dengue virus replication in a polyploid mosquito cell culture grown in serum-free medium. **J. clin. Microbiol., 16:** 851-855, 1982.

9. KUNO, G; GOMEZ, I. & GUBLER, D.J. - Detecting artificial anti-dengue IgM immune complexes using an enzyme - linked immunosorbent assay. **Amer. J. trop. Med. Hyg., 36:** 153-159, 1987.

10. LI, F.-S.; YANG, F.-R.; SONG, J.-C.; GAO, H.; TANG, J.-Q.; ZOU, C.-H.; HU, B.-M.; WEN, S.-R. & IU, F.-X. - Etiologic and serologic investigations of the 1980 epidemic of dengue fever on Hainan Island, China. **Amer. J. trop. Med. Hyg., 35:** 1051-1054, 1986.

11. MARIANO, F. - A dengue. Considerações a respeito de sua incursão no Rio Grande do Sul em 1916. **Arch. bras. Med., 7:** 272-277, 1917.

12. MORENS, D.M.; RIGAU-PEREZ, J.G.; LOPEZ-CORREA, R.H.; MOORE, C.G.; RUIZ-TIBEN, E.E.; SATHER, G.E.; CHIRIBOGA, J.; ELIASON, D.A.; CASTA-VELEZ, A. & WOODALL, J.P. - Dengue outbreak investigation group dengue in Puerto Rico, 1977: public health response to characterize and control an epidemic of multiple serotypes. **Amer. J. trop. Med. Hyg., 35:** 197-211, 1986.

13. NOGUEIRA, R.M.R.; MIAGOSTOVICH, M.P.; LAMPE, E. & SCHATZMAYR, H.G. - Isolation of dengue virus type 2 in Rio de Janeiro. **Mem. Inst. Oswaldo Cruz, 85:** 253, 1990.

14. NOGUEIRA, R.M.R.; SCHATZMAYR, H.G.; MIAGOSTOVICH, M.P.; FARIAS, M.F.D.B. & FARIAS FILHO, J.C. - Virological study of dengue type 1 epidemic at Rio de Janeiro. **Mem. Inst. Oswaldo Cruz, 83:** 219-225, 1988.

15. OSANAI, C.H.; TRAVASSOS DA ROSA, A.P.A; TANG, A.T.; AMARAL, R.S.; PASSOS, A.D.C. & TAUIL, P.L. - Surto de dengue em Boa Vista, Roraima. **Rev. Inst. Med. trop. S. Paulo, 25:** 53-54, 1983.

16. PEDRO, A. - O dengue em Nictheroy. **Brasil-méd., 1:** 172-177, 1923.

17. PINHEIRO, F.P. - Dengue in the America, 1980-87. **Epidem. Bull., 10:** 1-8, 1989.

18. PINHEIRO, F.P.; SCHATZMAYR, H.G.; TRAVASSOS DA ROSA, A.P.A.; HOMMA, A. & BENSABATH, G. - Arbovirus antibodies in children of Rural Guanabara, Brazil. **Intervirology, 5:** 93-96, 1975.

19. RACE, M.W.; WILLIAMS, M.C. & AGOSTINI, C.F.M. - Dengue in the Caribbean: virus isolation in a mosquito (**Aedes pseudoscutellaris**) cell line. **Trans. roy. Soc. trop. Med. Hyg., 73:** 18-22, 1979.

20. REED, L.J. & MUENCH, H. - A simple method of estimating fifty per cent endpoints. **Amer. J. Hyg., 27:** 493-497, 1938.

21. REIS, T.J. - A febre dengue em Curityba. **Gaz. méd. Bahia, 4:** 263-266, 1896.

22. SCHATZMAYR, H.G.; NOGUEIRA, R.M.R. & TRAVASSOS DA ROSA, A.P.A. - An outbreak of dengue virus at Rio de Janeiro - 1986. **Mem. Inst. Oswaldo Cruz, 81:** 245-246, 1986.

23. SCHOEPP, R.J. & BEATY, B. - Titration of dengue viruses by immunofluorescence in microtiter plates. **J. clin. Microbiol., 20:** 1017-1019, 1984.

24. TESH, R.B. - A method for the isolation and identification of dengue viruses using mosquito cell cultures. **Amer. J. trop. Med. Hyg., 28:** 1053-1059, 1979.

25. VASCONCELOS, P.F.C.; TRAVASSOS DA ROSA, E.S. TRAVASSOS DA ROSA, J.F.S.; FREITAS, R.B. & TRAVASSOS DA ROSA, A.P.A. - Dengue 2 outbreak in Araguaina, Tocantins State, Brazil. In: **Virológica 91. Simpósio Internacional sobre Arboviroses dos Trópicos e Febres e Hemorrágicas**, Belém, 1991. **Resumos.** Belém, Pará, 1991. p. 3.

Recebido para publicação em 5/8/1992
Aceito para publicação em 15/10/1992