Rev. Inst. Med. Trop. Sao Paulo
54(1):49-51, January-February, 2012
doi: 10.1590/S0036-46652012000100009

BRIEF COMMUNICATION

FIRST ISOLATION OF DENGUE 4 IN THE STATE OF SÃO PAULO, BRAZIL, 2011

Iray Maria ROCCO(1), Vivian Regina SILVEIRA(1), Adriana Yurika MAEDA(1), Sarai Joaquim dos Santos SILVA(1), Carine SPENASSATTO(1), Ivani BISORDI(1) & Akemi SUZUKI(1)

SUMMARY

We report the first isolation of Dengue virus 4 (DENV-4) in the state of São Paulo, from two patients - one living in São José do Rio Preto and the other one in Paulo de Faria, both cities located in the Northwest region of the state. The virus isolations were accomplished in the clone C6/36 *Aedes albopictus* cell line, followed by indirect immunofluorescence assays, performed with type-specific monoclonal antibodies that showed positive reactions for DENV-4. The results were confirmed by Nested RT-PCR and Real-Time RT-PCR assays. The introduction of DENV-4 in a country that already has to deal with the transmission of three other serotypes increases the possibility of the occurrence of more severe cases of the disease. The importance of early detection of dengue cases, before the virus spreads and major outbreaks occur, should be emphasized.

KEYWORDS: Dengue 4; Virus isolation; RT-PCR.

Dengue is currently a major public-health problem, having become the most significant vector-borne viral disease worldwide[1]. Approximately 2 billion people live in risk areas, consisting mostly of tropical and subtropical developing countries, which means that 2/5 of world population is at risk of contracting dengue[6]. Dengue, in the Americas, has shown an upward trend, with more than thirty countries reporting cases of the disease[15].

Historically, references based on clinical signs for the presence of Dengue virus (DENV) in the 20th century in Brazil, date from 1916 in the state of São Paulo[7] and 1923 in the state of Rio de Janeiro (Niteroi)[13]. However, there is no laboratory diagnosis documentation. The virus reemerged in the North of Brazil in 1981, when an epidemic caused by DENV-1 and DENV-4 was registered in Boa Vista (RO)[12]. After that, a major outbreak caused by DENV-1 occurred in Rio de Janeiro in 1986[9]**.** The serotypes 2 and 3 were also introduced into the country in the following years through Rio de Janeiro, spreading to the other states where the vector *Aedes aegypti* has been present since its reintroduction in the late 70's[9,10].

Dengue transmission, with clinical and laboratory diagnosis, began to be reported in the state of São Paulo in March 1987[14]. In the summer of 1990/91 an epidemic of major proportions caused by DENV-1 started in Ribeirão Preto, and quickly spread to neighboring counties and other regions[2,14]. In 1997, a new serotype, DENV-2 was introduced and in 2002 the first autochthonous case of DENV-3 was reported[16]. Epidemics have occurred yearly in different regions of the state with transmission throughout the year, showing that the disease has become endemic in several counties[2].

In August 2010, the first cases of DENV-4 were registered in Brazil, in Boa Vista (RO)[17]. Subsequently, the virus was detected in the North (states of Amazonas and Pará) and in the Northeast (states of Bahia, Pernambuco and Piauí)[8]. In the Southeast, the first episode of the disease occurred in the state of Rio de Janeiro in 2011[11].

In the present study, we report the first isolations of DENV-4 in the state of São Paulo, from two patients - one living in São José do Rio Preto and the other one in Paulo de Faria, both located in the Northwest region of the state. The onset of the symptoms occurred on 27th February 2011 for the patient from São José do Rio Preto (SPH 317947) and 4th March 2011 for the patient from Paulo de Faria (SPH 319325). The symptoms shown by the patients were fever, headache and myalgia, compatible with the classification of Dengue Fever. The evolution of the disease was satisfactory, with complete recovery.

Virus isolations were accomplished in clone C6/36 *Aedes albopictus* cell line, inoculated with serum samples collected from both patients on the day one after the onset of symptoms. Indirect immunofluorescence assays, performed with type-specific monoclonal antibodies[3], showed

(1) Núcleo de Doenças de Transmissão Vetorial/Instituto Adolfo Lutz, São Paulo, SP, Brazil.
Correspondence to: Iray Maria Rocco, Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brazil. E-mail: imrocco@uol.com.br

Table 1
Analysis of samples from two patients with suspected DENV infection

Samples	Age/ Sex	County	Isol. Cel.	Nested RT-PCR (bp)*	Real-Time RT-PCR (Cp)**	GenBank accession nº.
SPH 317947	31/F	São José do Rio Preto	DENV-4	DENV-4 (392)	DENV-4 (25)	JN092554
SPH 319325	49/M	Paulo de Faria	DENV-4	DENV-4 (392)	DENV-4 (31)	JN092556

* bp: base pair. **Avg Cp: Average of crossing point cycle threshold, automatically calculated by the LightCycler 480 II software (Roche Applied Science).

a positive reaction for DENV-4. Negative and positive controls were used in the tests.

The results were confirmed by conventional Nested Reverse Transcriptase Polymerase Chain Reaction (Nested RT-PCR) and Real-Time RT-PCR assays. Briefly, viral RNA was extracted either from the patient's serum or the supernatant of the C6/36 infected cells by using QIAamp Viral RNA Mini kit (QIAGEN, Valencia, CA, USA). Nested RT-PCR was performed employing the protocol described by LANCIOTTI *et al.,* 1992[6]; the Real-Time RT-PCR in a fourplex format was accomplished using the protocol described by JOHNSON *et al.,* 2005[5] protocol, in a LightCycler[R] 480 II System (Roche Applied Science). The crossing point cycle threshold values (C_p) for this assay are shown in Table 1. The samples were determinated to be positives if the C_p value was ≤ 36. Agarose gel analysis of the DNA product from dengue viruses are shown in Fig. 1. Nested RT-PCR products were purified and directly sequenced using Big Dye v.3.3 terminator chemistry. Sequences were determined using the Applied Biosystems 3130XL DNA sequencer. The nucleotide sequences of capsid-prM junction for the DENV-4 serotype, generated for this study, are deposited in GenBank under accession numbers JN092554 (SPH 317947) and JN092556 (SPH 319325).

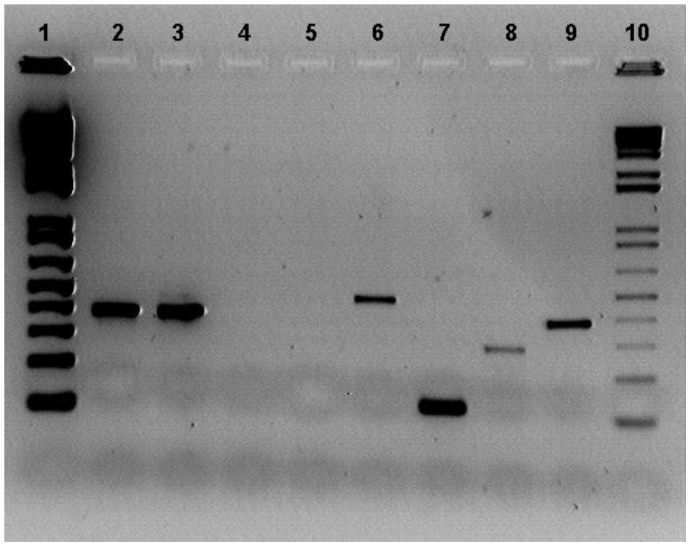

Fig. 1 - Agarose gel analysis of Nested RT-PCR products from Dengue virus. Lane 1 and 10: 1 kb Plus ladder (Invitrogen); Lane 2: sample patient - SPH 319325 (392 bp); Lane 3: sample patient - SPH 317947 (392 bp); Lane 4 and 5: negative control; Lane 6: positive control DENV-1 (482 bp); Lane 7: positive control DENV-2 (119 bp); Lane 8: positive control DENV-3 (290 bp); Lane 9: positive control DENV-4 (392 bp).

São José do Rio Preto is situated 454 km from the capital of the state and has a population of 408,435 inhabitants[4]. The city is a great industrial, cultural and service center in the region, being cut by two major and important highways[4].

Paulo de Faria is a small town of 8,589 inhabitants[4], 540 km away from the capital of the state. It is situated on the banks of the River Grande (Água Vermelha dam) on the border of the State of Minas Gerais[4]. As this area is surrounded by rivers, it attracts many people interested in fishing.

For all these reasons the presence of DENV-4 in this region is a cause for concern since there is a massive number of people and also because several epidemics of serotypes 1, 2 and 3 have already occurred. In addition, as DENV-4 has not circulated in Brazil for almost 30 years, the majority of the population is vulnerable to it. It is essential to use rapid and sensitive laboratory diagnostic techniques to ensure early detection of cases.

The introduction of DENV-4 into a country that already had to deal with the transmission of the other three serotypes increases the possibility of occurrence of more severe cases of the disease. The importance of early detection of dengue cases, before the virus spreads and major outbreaks occur, should be emphasized.

RESUMO

Primeiro isolamento de Dengue 4 no Estado de São Paulo, Brasil, 2011

Relatamos o primeiro isolamento do vírus Dengue 4 (DENV-4) no Estado de São Paulo, de dois pacientes residentes em São José do Rio Preto e Paulo de Faria, ambos municípios localizados na região Noroeste do Estado. O isolamento do vírus foi realizado em clone C6/36, linhagem de células de *Aedes albopictus* seguido por imunofluorescência indireta, realizada com anticorpos monoclonais tipo específicos, que apresentou reação positiva para DENV-4. Os resultados foram confirmados por testes de *Nested* RT-PCR e RT-PCR em Tempo Real. A introdução do DENV-4 no país, com uma população suscetível a esse vírus e que já convive com a transmissão de outros três sorotipos, aumenta a possibilidade da ocorrência de casos mais graves da doença. Deve ser enfatizada a importância da detecção precoce de casos de dengue, antes que ocorra a propagação do vírus e que surtos importantes aconteçam.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Cecília Luiza S. Santos for the critical

reading of the manuscript and suggestions and to Marcia Cristina Toledo Silva of the Núcleo de Cultura de Células for supplying the cell cultures.

REFERENCES

1. Azevedo RSS, Martins LC, Rodrigues SG, Travassos da Rosa JFS, Vasconcelos PFC. Arboviroses. In*:* Farhat CK, Carvalho LHFR, Succi RCM, coordenadores. Infectologia pediátrica. 3ª ed. São Paulo: Atheneu; 2007. p. 533-51.

2. Glasser CM, Pereira M, Katz G, Kavakama BB, Souza LTM, Ferreira IB, *et al.* Dengue no Estado de São Paulo: exemplo da complexidade do problema neste final de século. Revista CIP. 1999;2(4). Available from: http://www.cip.sp.gov.br/revistac4.htm

3. Gubler DJ, Kuno G, Sather GE, Velez M, Oliver A. Mosquito cell culture and specific monoclonal antibodies in surveillance for dengue viruses. Am J Trop Med Hyg. 1984;33:158-65.

4. IBGE: Instituto Brasileiro de Geografia e Estatística. Censo 2010. Available from: http://www.censo 2010.ibge.gov.br

5. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol. 2005;43:4977-83.

6. Lanciotti RS, Calisher CH, Gubler DJ, Chang G-J, Vorndam V. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992;30:545-51.

7. Meira R. "Urucubaca", gripe ou dengue? Dengue. In: Clínica Médica. São Paulo: Gráfica O Estado de S. Paulo; 1916. p. 273-85.

8. Ministério da Saúde. Secretaria de Vigilância em Saúde. Balanço Dengue. Informes Técnicos. 2011;1:1-12. Available from: http://portal.saude.gov.br/portal/saude/profissional/area.cfm?id_area=1525.

9. Nogueira RMR, Miagostovich MP, Schatzmayr HG, dos Santos FB, de Araujo ES, de Filippis AM, *et al.* Dengue in the state of Rio de Janeiro, Brazil 1986-1998. Mem Inst Oswaldo Cruz. 1999;94:297-304.

10. Nogueira RMR, Miagostovich MP, de Filippis AMP, Pereira MA, Schatzmayr HG. Dengue virus type 3 in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2001;96:925-6.

11. Nogueira RMR, Eppinghaus AL. Dengue virus type 4 arrives in the state of Rio de Janeiro: a challenge for epidemiological surveillance and control. Mem Inst Oswaldo Cruz. 2011;106:255-6.

12. Osanai CH, Travassos da Rosa AP, Tang AT, Amaral RS, Passos AD, Tauil PL. Surto de dengue em Boa Vista, Roraima. Rev Inst Med trop Sao Paulo. 1983;25:53-4.

13. Pedro A. O dengue em Nictheroy. Brazil Médico. 1923;37:173-7.

14. Rocco IM, Ferreira IB, Katz G, Souza LTM, Kimura-Gushiken EK, Mendes KHC, *et al.* Ocorrência de dengue no Estado de São Paulo, Brasil, de 1986 a 1996. Rev Inst Adolfo Lutz. 1998;57:7-12.

15. San Martín JL, Brathwaite O, Zambrano B, Solórzano JO, Bouckenooghe A, Dayan GH, *et al.* The epidemiology of dengue in the Americas over the last three decades: a worrisome reality. Am J trop Med Hyg. 2010;82:128-35.

16. Santos CLS, Sallum MAM, Foster PG, Rocco IM. Molecular analysis of the dengue virus type 1 and 2 in Brazil based on sequences of the genomic envelope-nonstructural protein 1 junction region. Rev Inst Med Trop Sao Paulo. 2004;46:145-52.

18. Tinhorão JG, Penna GO, Carmo EH, Azevedo RSS, Nunes MRT, Vasconcelos PFC. Dengue virus serotype 4, Roraima State, Brazil. Emerg Infect Dis. 2011;17:938-40.

Received: 08 June 2011
Accepted: 19 December 2011