Rev. Inst. Med. trop. S. Paulo
41 (3): 207-208, May-June, 1999.

BRIEF COMMUNICATION

DETECTION AND IDENTIFICATION OF DENGUE-2 VIRUS FROM SANTA CRUZ-BOLIVIA BY A SINGLE TUBE RT-PCR METHOD

Carlos PEREDO(1), Tania GARRÓN(1), Jose Luis PELEGRINO(2), Eva HARRIS(3) & Alberto GIANELLA(1)

KEYWORDS: Dengue; RT-PCR; Bolivia.

Dengue virus type 1 was introduced to Brazil in 1986 where it produced outbreaks in several states[5]. In August of 1987 it made its first appearance in Santa Cruz-Bolivia where the infestation rate by *Aedes aegypti* was over 20%[7]. In that first Bolivian Dengue outbreak, the only serotype isolated in C6/36 mosquito cells by the CDC[4] was Dengue virus type 1, bearing the same genotype as the one isolated in Brazil. No cases of DHF nor fatalities were officially reported in Bolivia. Although only 6841 cases were clinically reported, due to the lack of diagnostic capabilities in Bolivia, the WHO[6] estimated that the real number of people infected by the virus was over 200,000 (50% of the population in Santa Cruz in that time).

In the next 9 years, no Dengue cases were reported although there is no reason to believe the virus was not present, particularly, due to the clinically over-reported cases of Mayaro infection in the city (never proven laboratorially). In March of 1996, the Centro Nacional de Enfermedades Tropicales (Cenetrop) established the most needed serological test (Mac Elisa) to start testing these supposed Mayaro cases in a Dengue surveillance net established in several health Centers scattered throughout the city. Out of 238 samples with clinical compatibility to Dengue symptoms processed in that year, 55 (23.1%) were in fact recent Dengue infection and the infestation rate by *Aedes aegypti* was 18% in the city. In 1997, the number of sera processed until August was 489 with 221 positive (45.2%). The IgMs were detected in patients from Santa Cruz de la Sierra, Cotoca, Porongo, Montero, El Torno, Minero, Roboré and San Ignacio all in Santa Cruz State.

Due to the lack of capabilities to do virus isolation in tissue culture and RT-PCR at the time, 14 acute sera were sent to the CDC and Fiocruz. Both places were able to isolate Dengue virus type 2 in 4 (28.6%) sera. No other serotype was isolated. The genotype turned out to be Jamaica which is known for having caused DHF in countries where it has been detected[1].

In 1997, Cenetrop conducted a random epidemiological study in Santa Rosita, a neighborhood in Santa Cruz representative to the entire city in order to estimate the Dengue prevalence . A total number of 445 sera were processed by Mac Elisa according to KUNO *et al.*[3] with 19 positive cases (4.3%). This indicates that ca. 43,000 of the Santa Cruz de la Sierra inhabitants had been in contact with the virus in the last three months. In the same study, 60 acute sera with fever up to 5 days prior to the blood collection were obtained. Although these acute sera were negative when processed by Mac Elisa, they were also processed by the recently established RT-PCR and C6/36 Tissue Culture technology in Cenetrop.

The enzymatic amplification of a segment of DNA is obtained in the RT-PCR technique by the simultaneous primer extension of complementary strands of DNA. This DNA , which is amplified is the result of a previous process of reverse transcription from the viral RNA.

The single tube RT-PCR protocol used is the one described by HARRIS in which a concensus primer for all dengue virus is combined with serotype specific primers, which are: Concensus primer (EHD1) = 5'-TCAATATGCTGAAACGCGCGAGAAACCG-3', Dengue-1 specific primer (DTS1) = : 5'-CGTCTCAGTTGATCCGGGGG-3', Dengue-2 specific primer (DTS2) = 5'-CGCCACAAGGGCCATGAACAG-3', Dengue-3 specific primer (DTS3) = 5'-TAACATCATCATGAGACAGAGC-3' and Dengue-4 specific primer (DEN 4) = 5'-TGTTGTCTTAAACAAGAGAGGTC-3'. The reaction mixture contains:

Reagents	Description	Final concentration/ 25 uL
Buffer 10 X + T	Dengue + Triton	1 X
dNTPs	5 mM A,C,G T	0.2 mM c/u
Primer 1	EHD1	1.0 uM

(1) Centro Nacional de Enfermedades Tropicales (Cenetrop). Casilla Postal 2974, Santa Cruz, Bolivia.
(2) Instituto "Pedro Kourí" (IPK), Box 601 Marianao13, Ciudad de la Habana, Cuba.
(3) University of California at San Francisco (UCSF). Box 0422, 94143-0422 San Francisco, Ca, USA.
Correspondence to: Dr. Alberto Gianella, Centro Nacional de Enfermedades Tropicales (CENETROP), Casilla 2974, Santa Cruz, Bolivia. Email: cenetrop@3millenium.com; Fax 591. 3. 54 18 01

Primer 2	DTS1	1.0 uM
Primer 3	DTS2	0.5 uM
Primer 4	DTS3	0.5 uM
Primer 5	DEN4	1.0 uM
DTT		5 mM
TMAC		30 mM
Betaine		0.5 M
dd H2O	DEPC - dd H2O	To complete 25 ul
Taq polymerase	Taq polymerase	0.025 U/ uL
Transcriptase reverse	rav-2	0.025 U/ uL

The amplification consists of 40 cycles each one at 94 °C for 30 s, at 55 °C for 1 min and at 72 °C for 2 min, this is preceded by an initial reverse transcription reaction at 42°C for 1 h and followed by a final extention at 72 °C for 5 min.

Fig. 1 - Gel showing in dwell 10, a RT-PCR amplified Dengue 2 virus cDNA from patient from Santa Rosita, Santa Cruz.

1 = Molecular weight marker; (2-6 = controls): 2 = D1, 3 = D2, 4 = water, 5 = D3, 6 = D4; 7-10 samples. 10 = D2 in patient.

The size of the amplified products are:

482-basepair (Dengue-1; EHD1 - DTS1)

119-basepair (Dengue-2; EHD1 - DTS2)

290-basepair (Dengue-3; EHD1 - DTS3)

389-basepair (Dengue-4; EHD1 - DEN4)

(Modification of LANCIOTTI *et al*. (1993) **J. clin. Microbiol., 30:** 545-551).

Out of the 60 sera processed, one (1.6%) amplified a band corresponding to the Dengue virus type 2 (See Fig. 1). The patient was a 34 year-old woman who had had fever for less than 6 days before the sample was taken. She had developed the typical symptomatology of Dengue: rash, nausea, retro-orbital pain, headache, myalgia, arthralgia and backache.

This is the first case of dengue virus isolation and identification ever performed in Bolivia. This serum was confirmed by inoculation in C6/36 mosquito cells according to the protocol by KUNO *et. al*.[2]. Molecular characterization of the isolated dengue virus type 2 is in progress.

ACKNOWLEDGMENTS

This project is funded by the Kingdom of Belgium (AGCD) and by the Program of Applied Molecular Biology/Appropriate Technology Transfer (AMB/ATT) University of California at San Francisco.

REFERENCES

1. GUBLER, D.J. - Emergence of epidemic dengue/dengue hemorrhagic fever as a global public health problem. In: INTERNATIONAL SEMINAR ON DENGUE, 1., Rio de Janeiro, 1996. **Summaries.** p. 13.

2. KUNO, G.; GUBLER, D.J.; VÉLEZ, M. & OLIVER, A. - Comparative sensitivity of three mosquito cell lines for isolation of dengue viruses. **Bull. Wld. Hlth. Org., 63:** 279-286, 1985.

3. KUNO, G.; GOMEZ, I. & GUBLER, D.J. - An ELISA procedure for the diagnosis of dengue infections. **J. virol. Meth., 33:** 101-113, 1991.

4. LANCIOTTI, R.S. - Molecular evolution and philogeny of the four serotypes of dengue viruses. In: INTERNATIONAL SEMINAR ON DENGUE, 1., Rio de Janeiro, 1996. **Summaries.** p. 15.

5. NOGUEIRA, R.M.; 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.

6. PINHEIRO, F.P. - Dengue in the Americas. 1980-1987. **Epidem. Bull., 10:** 1-8, 1989.

7. TÓRREZ, R. - Departamental head of vector control, 1997. (Personal communication).

Received: 15 October 1997
Accepted: 10 June 1999