

## DENGUE VIRUS TYPE 3 IN BOA VISTA, NORTHERN BRAZIL

*Dengue virus tipo 3 em Boa Vista, norte do Brasil*

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### RESUMO

Casos de doença febril aguda ocorridos em Boa Vista, estado de Roraima, Brasil foram investigados em julho de 2005. Amostras de sangue (n=142) foram obtidas de pacientes clinicamente suspeitos de febre do dengue (FD). A tentativa de isolamento viral foi realizada em células C6/36 para pacientes com menos de cinco dias de doença (n=96). As cepas isoladas foram identificadas pelo teste de imunofluorescência indireta (IFI) utilizando anticorpos monoclonais, bem como pela técnica de RT-PCR. Amostras de soro foram testadas pelo método de inibição da hemaglutinação (IH) e confirmadas pelo IgM-ELISA. O vírus dengue 3 (VDEN-3) foi isolado e detectado por RT-PCR em 41 pacientes. A região E de sete cepas foi seqüenciada, sendo as mesmas identificadas como pertencentes ao genótipo III. Pelo teste de IH, 98 amostras de soros foram positivas para anticorpos IH para o vírus da Dengue, sendo os resultados confirmados pela detecção de anticorpos IgM para dengue. Clinicamente, todos os

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pacientes mostraram quadro de FD, sendo todas as faixas etárias afetadas independentes do sexo. A epidemia de dengue ocorrida em Roraima foi causada pelo VDEN-3, genótipo III, genótipo este que circula nas Américas desde 1994.

#### PALAVRAS-CHAVE

Febre da dengue, dengue, genótipo, Brasil

#### ABSTRACT

An acute febrile illness in Boa Vista, Roraima state, Brazil, was investigated in July 2005. Blood samples (n=142) were obtained from patients with a clinical picture of Dengue fever (DF). Virus isolation was attempted for patients with less than 5 days of illness (n=96) into C6/36 cells. Isolates were typed using monoclonal antibodies in indirect fluorescent assay (IFA) and also by RT-PCR. Serum samples were screened by hemagglutination-inhibition (HI) and confirmed by IgM-ELISA. Dengue 3 (DENV-3) was isolated and detected by RT-PCR from 41 patients. The E region of seven isolates was sequenced and characterized as genotype III. By HI, 98 serum samples showed dengue antibodies which were confirmed by detection of anti-dengue IgM. Clinically all patients presented DF and all age groups were affected in both sexes. The outbreak of dengue in Boa Vista was caused by DENV-3, genotype III, similar to viral genotype circulating in the Americas since 1994.

#### KEY WORDS

Dengue fever, dengue, genotype, Brazil

## 1. INTRODUCTION

Dengue is an acute arboviral infectious disease caused by the dengue virus (DENV), genus *Flavivirus*, family *Flaviviridae*. There are four serotypes DENV-1, DENV-2, DENV-3, and DENV-4 which are transmitted to human by the bites of infected mosquitoes of the *Aedes* genus. Dengue is considered the most important arthropod-borne virus disease distributed in the World. In fact, dengue is endemic in four of the five continents, where approximately 2.5 billion people are under risk of dengue and according to World Health Organization, about 50 million people are infected worldwide each year (WHO, 1997; 2001; PAHO, 2003).

Clinically, dengue presents several forms including asymptomatic or unapparent infection, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (WHO, 1997). The illness begins suddenly with high fever ( $>39^{\circ}\text{C}$ ) accompanied of headache, muscle and joint pains, dizziness, anorexia, retro bulbar pain, nausea, vomiting, skin rash, among others. In severe forms, such as DHF and DSS, it is usually also common the occurrence of hepatomegaly, splenomegaly, abdominal pain, hypotension and shock (Halstead, 1981; Kouri *et al.*, 1989; Gubler, 1998). Case fatality rate in dengue severe forms ranges from 0.1 to 5% (WHO, 1997; Pinheiro & Nelson, 1997).

This study was undertaken to define the causal agent of an acute febrile dengue-like disease occurred in Boa Vista, Roraima State, Brazil, in July 2005. A team of the Instituto Evandro Chagas performed clinical, epidemiological and laboratorial studies with logistic support of the Prefeitura Municipal de Boa Vista.

## 2. MATERIAL AND METHODS

### 2.1. STUDY SITE OR STUDY POPULATION

The current study was conducted in the city of Boa Vista (2° 51'N; 60° 43'W), capital of the Brazilian state of Roraima. The most recent census estimate indicates that approximately one third of the population (249,665) lives in the state capital (2006 census – available at [www.ibge.gov.br](http://www.ibge.gov.br)).

### 2.2. SAMPLES

During the period of 1 to 12 of July 2005, a total of 142 serum samples from people reporting acute febrile illness were obtained in the State and Municipality Health Units, in Boa Vista. In the same areas, diurnal mosquito captures were performed.

### 2.3. CELLS CULTURE AND VIRUS TYPING

All acute phase (reporting up to 5 days after the onset of the illness) blood samples (n=96) and mosquito lots were inoculated into C6/36 mosquito cells in attempts of virus isolation, as described elsewhere (Tesh, 1979). Ten day later, cultures showing or not cytopathic effect (CPE) were further used in indirect fluorescent assay (IFA) using monoclonal antibodies to identify the serotype responsible for patient's disease.

### 2.4. MOLECULAR BIOLOGY STUDIES

Viral RNA was extracted from the isolates directly from the supernatant of the infected cells using a commercial kit (Qiagen) or the Trizol reagent (Invitrogen). A standard RT-PCR protocol (Lanciotti *et al.*, 1994) was used for synthesis of the complimentary DNA (cDNA) using two sets of primers E953F/E1817R and E1794F/E2492R (Table 1) specific to DENV-3 E region. The RT-PCR amplicons were sequenced in the automated sequencer model ABI 377 (Applied Biosystems) using the dideoxy chain terminator method (Saitou & Nei, 1987; Sanger *et al.*, 1977) and the same set of primers. The nucleotide sequences obtained were aligned using the Clustal-W software and then, aligned sequences were used to construct phylogenetic trees using the neighbor-joining (NJ)

method (Felsenstein, 1985). For NJ analysis, a distance matrix was calculated from the aligned sequences using the Kimura two-parameter formula. Bootstrap analyses using 1,000 replicates were used to place confidence values on groupings (Shope & Sather, 1979).

Table 1

Primers used for genome amplification by RT-PCR and sequencing of DENV-3.

Primer	Sequence (5'→3')	Position	Product size
E 953F	G G A A A C A G A G A T T T T G T G G A	953-972	
E 1817R	G C A C A T T G C A T A G C T C A T C	1817-1837	882
E 1794F	T G G A C A A A T T G G A A C T C A A G G	1794-1814	
E 2492R	A A T T T G T A T T G C T C T G T C C A G G T	2492-2514	721

### 2.5. SEROLOGY

All serum samples obtained from patients were further submitted to hemagglutination-inhibition (HI) and IgM-ELISA (enzyme immuno-sorbent assay for IgM capture) procedures as described elsewhere (Kuno *et al.*, 1987; Vasconcelos *et al.*, 1997) aiming to confirm recent infections by dengue virus. Statistical analysis was performed with data obtained from clinical, epidemiological, and laboratorial investigation using the software Epi-Info 6.02. Statistical significance was considered when  $p < 0.05$ .

### 3. RESULTS

Of all sera collected ( $n=142$ ), 73 (51.4%) were positive by serologic testing or DEN virus isolation. Serologically, by HI 98 (69%) samples were positive for dengue primary or secondary responses and confirmed by IgM-ELISA by the presence of IgM antibodies. Furthermore, from 96 samples submitted for virus isolation attempt, in 41 (42.7%) the DENV-3 was isolated.

Among the patients examined 70 (49.3%) were male and 72 (50.7%) female. No statistically significant was observed difference in the frequency between sexes. Patients with dengue confirmed presented the classic dengue fever, the most prevalent symptoms were severe headache, nausea-vomiting, and retro-bulbar pain. Other symptoms reported include malaise, myalgia, arthralgia, and anorexia. Signs of plasma leakage or hemorrhagic diathesis were not observed.

The distribution of isolates by days of sickness has showed that strains were isolated up to five days from the onset (Figure 1). All age groups were infected, but people between 15-24, 25-34, and 35-44 years of age were the most affected (Figure 2).

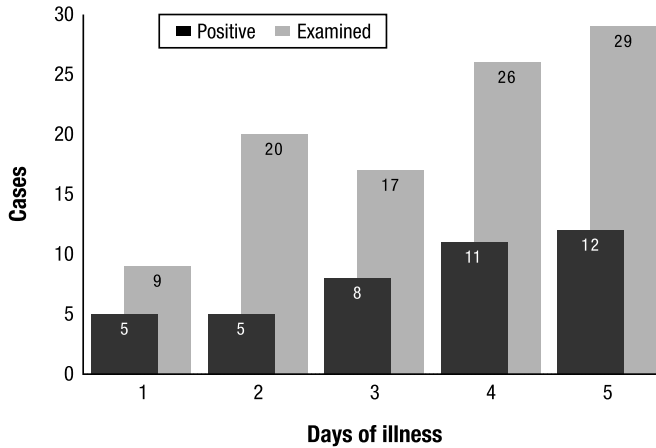


Figure 1

Dengue fever outbreak in Boa Vista, Roraima, 2005. Attempts of virus isolation according of the days of illness.

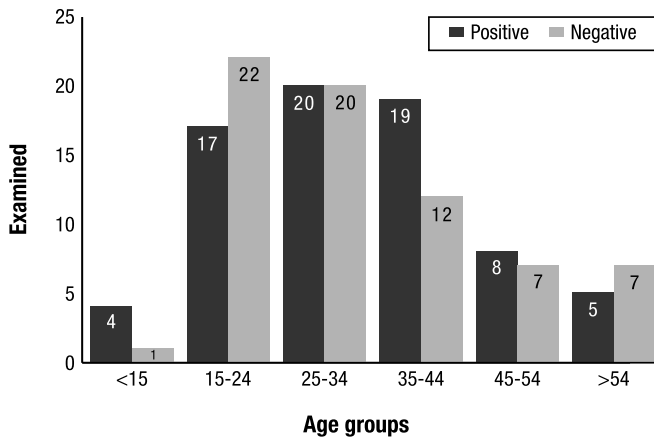


Figure 2

Distribution of results by age groups of serologic MAC-ELISA for IgM anti-dengue in blood samples of patients from Boa Vista, Roraima, 2005.

An isolation of DENV-3 was also obtained from a lot of female *Aedes aegypti* captured in the São Pedro District, among 847 females collected which were sorted into 33 inoculated lots. All isolates from patients and from *Aedes aegypti* typed as DENV-3 were later molecularly characterized as belonging to the genotype III (Figure 3).

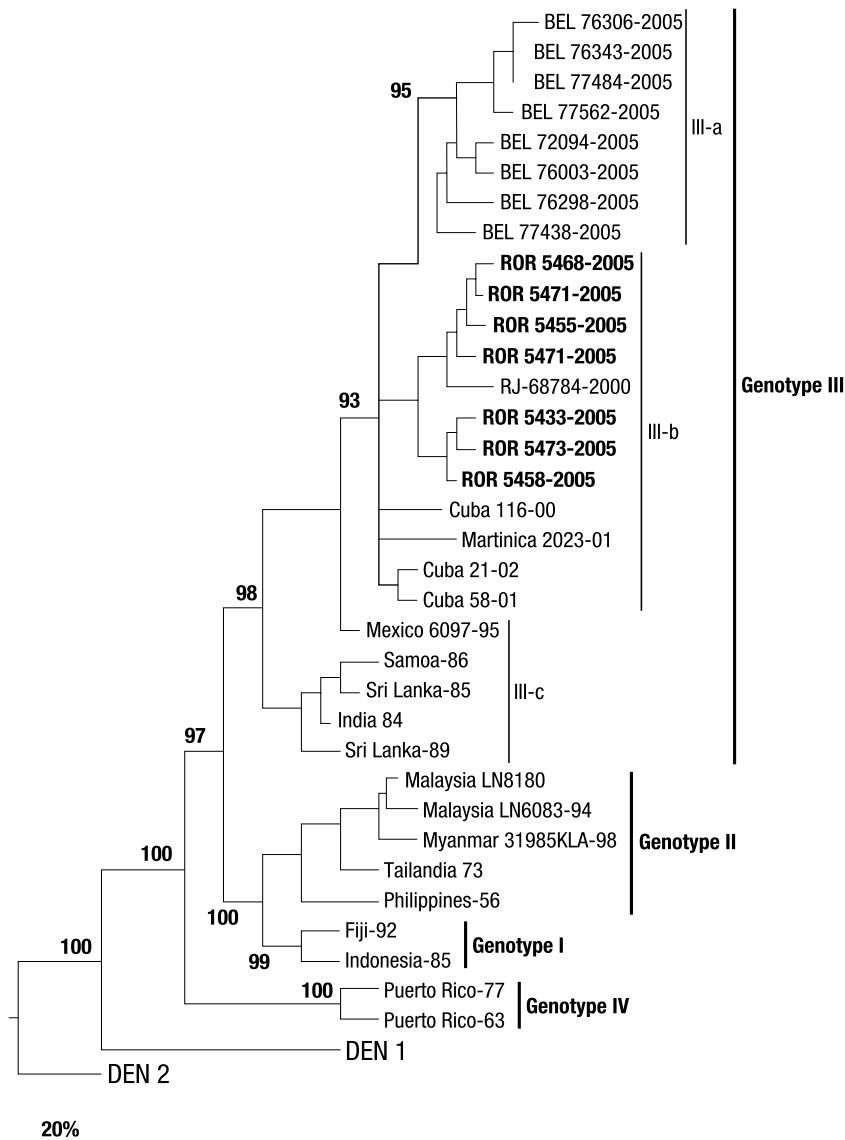


Figure 3

Neighbor-joining phylogenetic tree based on E nucleotide sequences of representative members of DEN-3 genotypes I to IV, including isolates from Boa Vista, Roraima State (ROR), 2005 which appear in bold. All ROR strains were genotyped as members of genotype III. Numbers adjacent to each clade represent the percentage bootstrap support calculated for 1,000 replicates. DEN-1 and DEN-2 viruses were used to root the tree. The scale bar represent 20% nucleotide divergence.

#### 4. DISCUSSION

The first dengue outbreak diagnosed in Brazil just occurred in Boa Vista, Roraima in 1981/82 when an explosive dengue fever epidemic caused by serotypes DENV-1 and DENV-4 were firstly isolated in Brazil (Vasconcelos *et al.*, 1999). Then, dengue was only reported in Roraima in the 1990's after the reintroduction and widespread of dengue in Brazil (Vasconcelos *et al.*, 1999; Travassos da Rosa *et al.*, 2000; Schatzmayr *et al.*, 1986; Uzcategui *et al.*, 2003; Siqueira Jr. *et al.*, 2005). Although the three serotypes circulating in Brazil have been isolated in Boa Vista, DENV-1, DENV-2, and DENV-3, DHF cases have not been recognized in Roraima, despite to be neighbor of Venezuela where DHF/DSS has been frequently diagnosed (Pinheiro & Nelson, 1997; Schatzmayr *et al.*, 1986; Vasconcelos *et al.*, 1999).

In fact, in Boa Vista all patients reported an acute picture typical of DF. Hemorrhagic symptoms or alert signs were not observed and for all patients recovery was uneventful. Despite dengue have been confirmed from patients from different age groups, disease was mostly diagnosed among young adults of both genders. Statistically significant differences were not observed among gender, but children and elderly were apparently little affected, although this finding could a consequence of sampling bias.

In the present outbreak, only DENV-3 was recovered from the blood of acute-phase patients. This found is very interesting since the widespread of serotype DENV-3 inside country was very fast and it has been found in almost all Brazilian states (Aquino *et al.*, 2006), despite this serotype was only few years ago introduced in Brazil (Siqueira Jr. *et al.*, 2005). Moreover, the isolation of a DENV-3 strain from a pooled *Aedes aegypti* captured in the same municipality where human cases were reported reinforce that transmission in the area was performed by this mosquito species, and the transmission rate calculated was 3.03% which is extremely high (Ocazonez *et al.*, 2006).

DENV-3 isolates were phylogenetically characterized as genotype III and as observed in Figure 3, all Roraima strains were too similar to the Brazilian topotype (RJ 68784) isolated in 2000 in the Rio de Janeiro State (Nogueira *et al.*, 2000; 2005), meaning that this serotype in Brazil has evolved very slowly, as expected. The nucleotide sequences of the Roraima state strains herein studied were deposited in GenBank with accession numbers from EU 145962 to EU145968.

Interesting, since its introduction in Brazil in 2000, DENV-3 has been the serotype mostly responsible for DHF/DSS during epidemics in several Brazilian states (Siqueira Jr. *et al.*, 2005; Aquino *et al.*, 2006), but this time in Boa Vista, none cases of DHF/DSS were reported, despite isolates from Boa Vista which were sequenced were similar (genotype III) to the ones associated with DHF/DSS (Figure 3).

To conclude, this study confirms that the 2005 DF outbreak in Boa Vista was caused by DENV-3, genotype III, related to those circulating in several areas in the Americas (Rodríguez-Roche *et al.*, 2005), including Mexico, Puerto Rico, The US Virgin Islands, Guadeloupe, Martinique, Belize, El Salvador, Costa Rica, Nicaragua, Colombia Ecuador, Paraguay, Venezuela, and Brazil (Siqueira Jr. *et al.*, 2005; Vasconcelos *et al.*, 1999; Travassos da Rosa *et al.*, 2000; Nogueira *et al.*, 2000; Aquino *et al.*, 2006) where it has been associated with DF and DHF/DSS.

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