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Genomic Characterization of Wild-Type Measles Viruses That Circulated in Different States in Brazil During the 1997 Measles Epidemic

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Despite the marked reduction in the incidence of measles in Brazil, a measles epidemic occurred in this country in 1997. The measles cases observed during this epidemic began to reappear in large numbers in São Paulo, and spread to Rio de Janeiro and other Brazilian states. In the present study molecular biology techniques were used for the detection and genomic characterization of measles viruses from clinical samples such as urine and nasopharyngeal secretions collected in the states of Rio de Janeiro, Minas Gerais and Paraná, during the 1997 epidemic. RT-PCR and nucleotide sequence analysis of part of the carboxyl-terminal region of the nucleoprotein gene of measles viruses obtained directly from clinical samples or from infected cell cultures during this epidemic classified all as wild-type of genotype D6. As the genotype D6 was identified in different Brazilian states, this study demonstrated that this genotype was circulating in Brazil during the 1997 epidemic. ***J. Med. Virol. 63:299–304, 2001.***

KEY WORDS: measles; Brazil; epidemic; RT-PCR; nucleotide sequence; nucleoprotein

INTRODUCTION

Measles is a disease characterized by high fever, cough, coryza, conjunctivitis and the appearance of a maculopapular rash. Measles virus, a highly contagious virus, is transmitted by droplets or airborne spray from the respiratory tract of infected individuals to mucous membranes in the upper respiratory tract or the conjunctivae of susceptible individuals [de-Quadros et al., 1996]. Despite the encouraging progress toward control of measles in several areas of the world by the WHO Expanded Program on Immunization (EPI), this disease still remains a significant health problem with outbreaks or epidemics still observed worldwide. Although measles virus is considered to be serologically monotypic, antigenic and genetic variability was demonstrated for wild-type measles viruses [Rota et al., 1995a, 1996; Bellini and Rota, 1998]. Studies have demonstrated that the nucleotide sequence encoding the carboxyl-terminal part of the nucleoprotein (N) is the most variable of genome, and suitable for strain characterization and molecular epidemiological studies Taylor et al., 1991; Rima et al., 1995; Rota et al., 1995a, 1996; Kreis et al., 1997; Bellini and Rota, 1998; Santibanez et al., 1999].

In 1992, the Ministry of Health in Brazil carried out a National Vaccination Campaign against measles, covering 48 million children from 9 months to 14 years of age, achieving a coverage rate over 95%. As a result of measles-control efforts, a substantial reduction in the incidence of the disease was observed in this country. Despite the efforts to eradicate the disease in Brazil, a measles epidemic occurred in 1997 with more than 53,000 confirmed cases reported. This epidemic began early in 1997 in the state of São Paulo, where more than 42,000 cases occurred. Most of the cases during this

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epidemic occurred in persons residing in the greater São Paulo metropolitan area. Many cases occurred among young adults who were members of groups congregating in enclosed environments, including male migrant workers from rural areas, students, health care workers, and military recruits [Center for Disease Control, 1998]. Genomic sequencing of virus isolates from São Paulo during this epidemic, carried out by Centers for Disease Control and Prevention, United States, demonstrated that the virus circulating in São Paulo was similar to virus isolates obtained recently in Europe, suggesting that the virus responsible for the epidemic may have been imported from Europe [Center for Disease Control, 1998]. The measles virus circulating in São Paulo spread to almost every other state in Brazil. Some other Brazilian states reporting large numbers of measles cases included Rio de Janeiro, Minas Gerais, Paraná, Bahia, Ceará, and the Federal District (unpublished results). In the present study nucleotide sequence analysis was undertaken in Brazil, on part of the nucleotides that code for the carboxyl terminus of the N gene of measles virus samples obtained in the states of Rio de Janeiro, Minas Gerais, and Paraná during this epidemic to identify the genotype of the viruses and to confirm their relation with the isolates from São Paulo.

METHODS

Viruses

The measles viruses analyzed in this study were obtained from nasopharyngeal secretions or urine collected in the states of Rio de Janeiro, Minas Gerais and Paraná during the measles epidemic that occurred in 1997 in Brazil. Epidemiological data related to the wild-type virus samples analyzed in this study are described in Table I. All specimens were collected from serologically confirmed measles cases (IgM positive). The serological tests were performed using the IgM EIA test from both CDC [Erdman et al., 1991] and a commercial kit (Behring Diagnostics GmbH, Marburg). An Epstein-Barr virus-transformed marmoset B lymphoblastoid cell line, B95a cells [Kobune et al., 1990], was used to isolate measles viruses from clinical specimens such as urine and nasopharyngeal secretions (Table I shows the passage history of the strains). Some of the MV samples analyzed in this study were also obtained directly from urine or nasopharyngeal secretions.

RNA extraction, cDNA synthesis, PCR and nucleotide sequence determination

The RNA from the viruses was extracted using TRIzol reagent (Gibco BRL, Life Technologies, Paisley, Scotland). After viral RNA extraction from culture tissue infected with urine or nasopharyngeal secretions or directly from urine or nasopharyngeal secretions, a 589 bp fragment of the carboxyl-terminus of the N protein was amplified by RT-PCR. For the RNA extracted from infected tissue culture, the PCR product with 589 bp was purified and subjected to nucleotide sequencing. For the RNA extracted directly from urine or nasopharyngeal secretions, a nested PCR was performed with three pairs of primers. The nested PCR products were then purified and also subjected to

TABLE I. Epidemiologic Data Related to the Measles Virus Samples Analysed in this Study*

Sample	Source of specimen	Age of patient	Vaccination status of the patient	Date of vaccination	Date of onset of rash	Collection date of specimen	Location/ city	Condition	Extent of measles activity in the area
376a/97	urine	8 mo	yes	06/17/97	06/14/97	06/18/97	Valença	measles	sporadic
376b/97	NPS								
376c/97	urine								
395/97	NPS	7 mo	no	—	06/25/97	06/26/97	Rio de Janeiro	measles/ pneumonia	sporadic
839/97	urine	32 y	NA	NA	08/11/97	08/13/97	Rio de Janeiro	measles	epidemic
902/97	urine	25 y	no	—	08/11/97	08/15/97	Rio de Janeiro	measles/ pneumonia	epidemic
427/97	urine	26 y	no	—	07/03/97	07/04/97	Angra dos Reis	measles	sporadic
485/97	NPS	40 y	yes	07/07/97	07/12/97	07/13/97	Angra dos Reis	measles	sporadic
3031/97	urine	NA	NA	NA	NA	09/24/97	Belo Horizonte	measles	epidemic
3032a/97	urine	39 y	no	—	09/20/97	09/20/97	Belo Horizonte	measles	epidemic
3032b/97	urine								
3039/97	NPS	27 y	yes	NA	03/10/97	03/10/97	Belo Horizonte	measles	epidemic
73/97	urine	28 y	no	—	20/11/97	24/11/97	Curitiba	measles	epidemic

*Valença, Rio de Janeiro and Angra dos Reis are cities that belong to the state of Rio de Janeiro.
Belo Horizonte is a city of the state of Minas Gerais.
Curitiba is a city of the state of Paraná. Na, data not available; NPS, nasopharyngeal secretion. The isolates 376c/97, 395/97, 839/97, 902/97 and 3032b/97 were passaged 1 time in B95a cells and isolates 485/97 and 3039/97 were passaged 2 times in B95a cells. The three cases reported as sporadic were the three first cases of each city in which epidemics later occurred.

nucleotide sequence analysis. The primers designed to amplify the variable region of the nucleoprotein (N) gene by RT-PCR, the reverse transcription (RT), the polymerase chain reaction (PCR) conditions, and the primers used for nucleotide sequence analysis were previously described [Rota et al., 1995b; Kreis et al., 1997]. Purification of PCR products, before nucleotide sequencing, was carried out using GlassMAX DNA Isolation Spin Cartridge System (Gibco BRL Life Technologies). Nucleotide sequencing of PCR products in both directions was performed using CircumVent Termal Cycle Dideoxy DNA Sequencing kit (New England Biolabs, Beverly, MA) and ATP S^{35} (Amersham, Buckinghamshire, UK).

RESULTS

In this study part of the N genes of samples obtained in the states of Rio de Janeiro, Minas Gerais and Paraná during the 1997 measles epidemic in Brazil, were sequenced to identify the genotype of the viruses. The nucleotide sequences (Fig. 1) obtained in this study were compared with a consensus sequence [Taylor et al., 1991; Rima et al., 1995], and with sequences of related

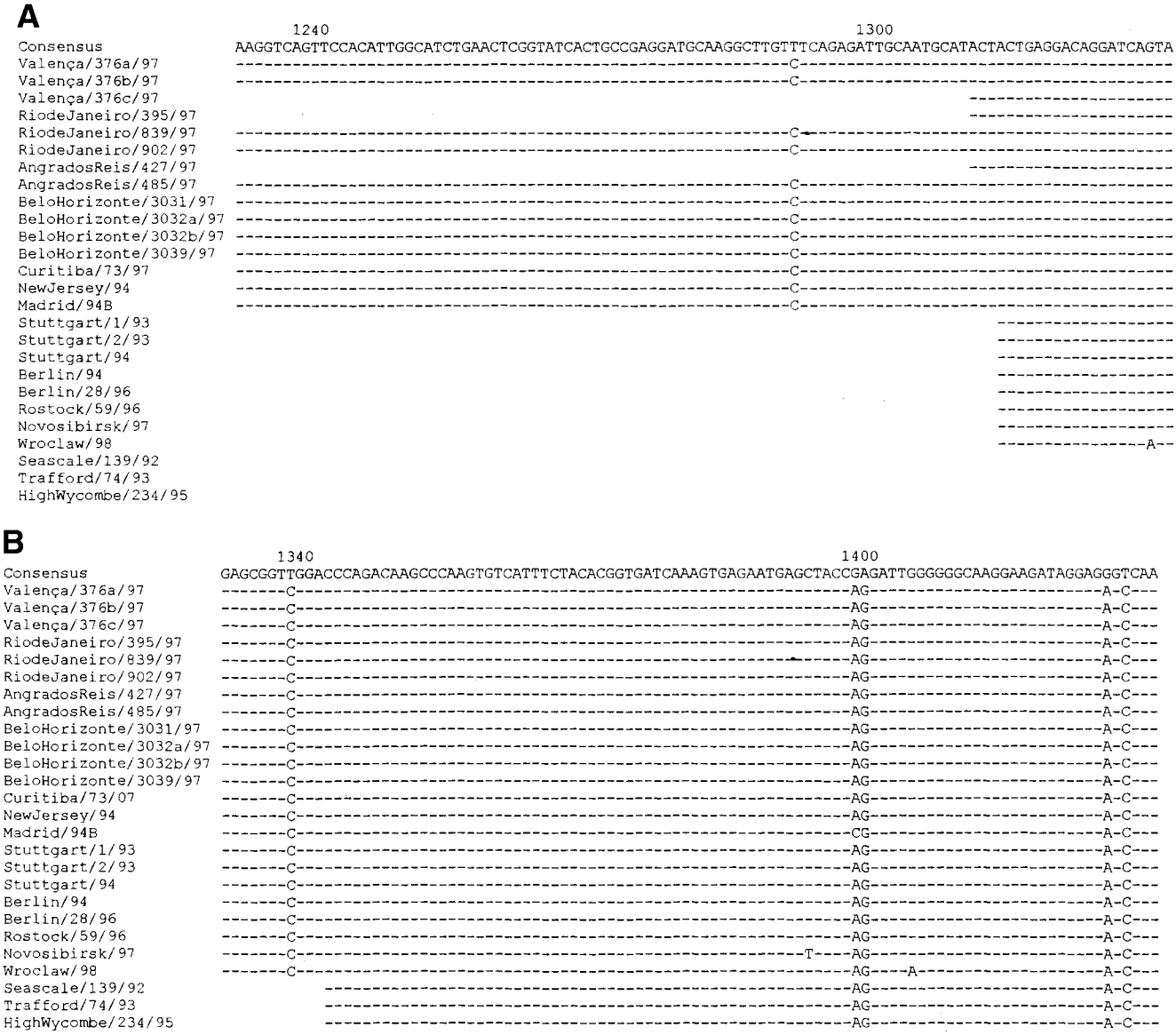

Fig. 1. Comparison of the nucleotide sequences of the hypervariable region of the N gene of different Brazilian measles virus isolates of the 1997 measles epidemic with a consensus sequence [Taylor et al., 1991; Rima et al., 1995], and sequences of other measles viruses obtained in other studies (see Table I). Differences from the consensus sequence are indicated. Numbering of the coding sequence of the N gene is according to Taylor et al. [1991] and Rima et al. [1995]. Samples analyzed directly from urine: 376a/97, 3031/97, 3032a/97 and 73/97. Samples analyzed directly from nasopharyngeal secretion: 376b/97 and 485/97. Samples analyzed after isolation in cell culture inoculated with urine: 376c/97, 839/97, 902/97, 427/97 and 3032b/97. Samples analyzed after isolation in cell culture inoculated with nasopharyngeal secretion: 395/97 and 3039/97.

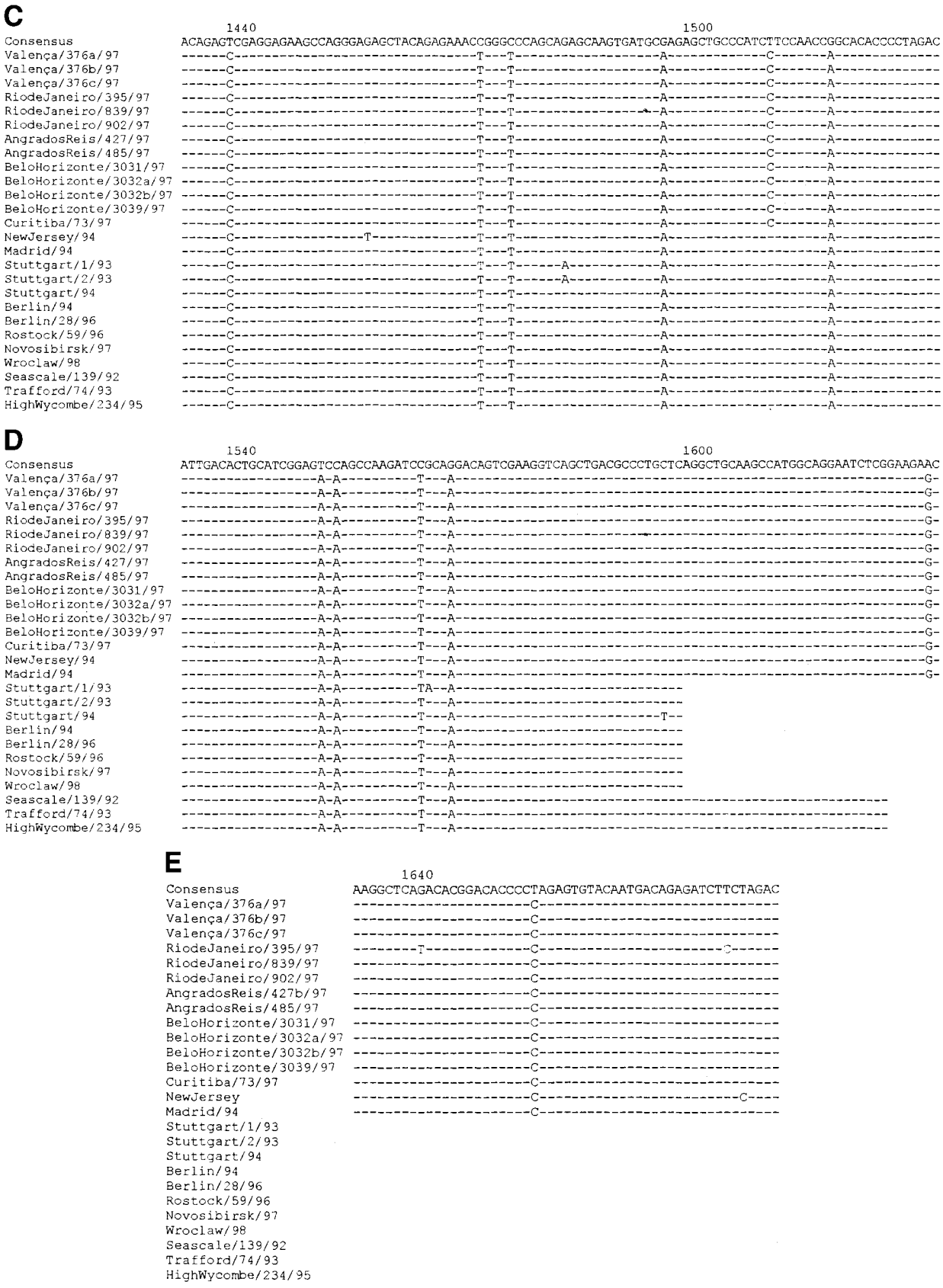

Fig. 1. (*Continued*)

TABLE II. MV Strains: Origin of Sequenced Measles Virus Samples Presented in This Study

Isolate	Location	Isolation year	Sequence reference	Accession number	Genotype
Valença/376a/97	Brazil	1997	this study	AJ272478	D6
Valença/376b/97	Brazil	1997	this study	AJ272479	D6
Valença/376c/97	Brazil	1997	this study	—	D6
Rio de Janeiro/395/97	Brazil	1997	this study	—	D6
Rio de Janeiro/839/97	Brazil	1997	this study	AJ272484	D6
Rio de Janeiro/902/97	Brazil	1997	this study	AJ272485	D6
Angra dos Reis/427/97	Brazil	1997	this study	—	D6
Angra dos Reis/485/97	Brazil	1997	this study	AJ272480	D6
Belo Horizonte/3031/97	Brazil	1997	this study	AJ272481	D6
Belo Horizonte/3032a/97	Brazil	1997	this study	AJ272482	D6
Belo Horizonte/3032b/97	Brazil	1997	this study	AJ272486	D6
Belo Horizonte/3039/97	Brazil	1997	this study	AJ272487	D6
Curitiba/73/97	Brazil	1997	this study	AJ272483	D6
New Jersey/94	USA	1994	Rota et al., 1996	L46750	D6
Madrid/94B	Spain	1994	Rima et al., 1995	X84863	D6
Trafford/74/93	UK	1993	Jin et al., 1997	U29327	D6
Seascale/139/92	UK	1992	Jin et al., 1997	U29284	D6
High Wycombe/234/95	UK	1995	Jin et al., 1997	U29302	D6
Stuttgart/1/93	Germany	1993	Santibanez et al., 1999	Y17029	D6
Stuttgart/2/93	Germany	1993	Santibanez et al., 1999	Y13825	D6
Stuttgart/94	Germany	1994	Santibanez et al., 1999	Y13823	D6
Berlin/94	Germany	1994	Santibanez et al., 1999	Y13814	D6
Berlin/28/96	Germany	1996	Santibanez et al., 1999	Y13815	D6
Berlin/3/96	Germany	1996	Santibanez et al., 1999	—	D6
Berlin/5/96	Germany	1996	Santibanez et al., 1999	—	D6
Berlin/9/96	Germany	1996	Santibanez et al., 1999	—	D6
Berlin/22/96	Germany	1996	Santibanez et al., 1999	—	D6
Berlin/50/96	Germany	1996	Santibanez et al., 1999	—	D6
Rostock/59/96	Germany	1996	Santibanez et al., 1999	Y13821	D6
Rostock/60/96	Germany	1996	Santibanez et al., 1999	—	D6
Rostock/61/96	Germany	1996	Santibanez et al., 1999	—	D6
Rostock/62/96	Germany	1996	Santibanez et al., 1999	—	D6
Rostock/69/96	Germany	1996	Santibanez et al., 1999	—	D6
Novosibirsk/97	Russia	1997	Santibanez et al., 1999	Y17032	D6
Wroclaw/1/98	Poland	1998	Santibanez et al., 1999	Y17026	D6
Wroclaw/2/98	Poland	1998	Santibanez et al., 1999	—	D6

measles viruses obtained in other countries (see Table II). Three of the samples analyzed (376a/97, 376b/97, 376c/97) of the state of Rio de Janeiro were from a patient with measles in the city of Valença. The nucleotide sequence of sample 376a/97 was obtained directly from urine, the nt sequence of sample 376b/97 was obtained directly from nasopharyngeal secretions, whereas the nt sequence of sample 376c/97 was of viruses isolated in cell culture inoculated with urine. All these three MV samples (376a, 376b and 376c) had the same nt sequence in the region analyzed and were of genotype D6. Another three sequences obtained (395/97, 839/97, 902/97) were of samples collected from three patients in Rio de Janeiro city, the capital of the state of Rio de Janeiro. The nt sequence of sample 839/97 was from a patient with measles, whereas the other two nt sequences, 395/97 and 902/97, were from samples collected from two patients with measles/pneumonia. The nucleotide sequences of the samples 839/97 and 902/97 were obtained of viruses isolated in cell culture inoculated with urine, whereas the nt sequence of sample 395/97 was obtained of viruses isolated in cell culture inoculated with nasopharyngeal secretions. Analysis of these three samples from the city of Rio de Janeiro demonstrated that they also belonged to genotype D6. Two of the samples analyzed (427/97 and 485/97) of the state of Rio de Janeiro were from two patients with measles in the city of Angra dos Reis. The nucleotide sequence of sample 427/97, was obtained of viruses isolated in cell culture inoculated with urine, whereas the nucleotide sequence of sample 485/97 was obtained directly from nasopharyngeal secretion. Analysis of the nucleotide sequence of these two samples (427/97 and 485/97) from Angra dos Reis showed that these two virus samples had the same nucleotide sequence, and belonged to genotype D6. Thus, the characterization of eight samples of the state of Rio de Janeiro demonstrated that they had the same nucleotide sequences and that all belonged to genotype D6.

The characterization of other three samples (3031/97; 3032/97; 3039/97) collected from three patients with measles in the city of Belo Horizonte, state of Minas Gerais, demonstrated that they had all the same nucleotide sequences and belonged also to genotype

D6. Two samples were analyzed from one patient: 3032a/97 was obtained directly from urine, whereas 3032b/97 was obtained of viruses isolated in cell culture inoculated with urine. The nucleotide sequence of sample 3031/97 was obtained of viruses isolated in cell culture inoculated with urine, whereas the nt sequence of sample 3039/97 was obtained of viruses isolated in cell culture inoculated with nasopharyngeal secretions. Analysis of another sample (73/97) collected from a patient with measles in the city of Curitiba, state of Paraná, demonstrated that the sample had the same nucleotide sequence of the viruses from the state of Rio de Janeiro and the state of Minas Gerais, belonging also to genotype D6. This study demonstrated that all the partially sequenced N gene segments from Brazilian measles viruses had the same sequence in the region analyzed (Fig. 1) and classified all as belonging to genotype D6.

DISCUSSION

The genotype D6 identified in the present study in the states of Rio de Janeiro, Minas Gerais and Paraná during the 1997 epidemic in Brazil, was also identified as the predominant genotype in the state of São Paulo during the same epidemic [Oliveira et al., 1998], confirming that the D6 genotype was circulating in different states in 1997 in Brazil. The previous genetic analysis of some measles virus samples isolated in Brazil in 1996 identified two other genotypes different from genotype D6 identified during the 1997 epidemic in Brazil. In 1996, genotype D5 was identified in some sporadic cases that occurred in the state of Bahia, whereas genotype C2 was identified in an outbreak that occurred in the state of Santa Catarina [Siqueira et al., 1999].

Molecular epidemiological investigations suggested that the D6 MV genotype detected first in São Paulo and then in Rio de Janeiro and other states, might have been imported from Europe [Center for Disease Control, 1998], where the D6 genotype is also widely distributed [Santibanez et al., 1999]. Comparison of the nucleotide sequences of MVs of genotype D6 obtained previously in Europe [Rima et al., 1995; Jin et al., 1997; Santibanez et al., 1999] and also in one case from United States [Rota et al., 1996] with those obtained for viruses in Brazil during the 1997 epidemic demonstrated a high degree of homology (Fig. 1) in the region analyzed. This study demonstrates that genetic analysis of MV is able to bridge information gaps in routine investigations and make possible to identify the transmission pathways of the viruses in Brazil.

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