Molecular surveillance of an imported measles virus infection in Sao Paulo, Brazil

Vírus do sarampo: vigilância molecular de um caso importado em São Paulo, Brasil

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RESUMO

No estado de São Paulo, Brasil, em função da eficiente estratégia para a vigilância do vírus do sarampo (VS), não houve registro de casos nativos de sarampo no período de 2001 a 2007. No estado de São Paulo foram registrados casos de sarampo importados, sendo 01 paciente em 2001, outro em 2002 e em 2005 foi alvo de investigação uma criança não vacinada, de 18 meses de idade com exantema e febre**,** que foi admitida em hospital privado. O Centro de Vigilância Epidemiológica descobriu que o irmão desta criança teve uma doença semelhante uma semana antes. A infecção pelo vírus do sarampo foi confirmada no Instituto Adolfo Lutz pela detecção de anticorpo IgM anti-VS, isolamento do vírus por meio de cultivo em células Vero/hSLAM e amplificação de RNA viral por RT-PCR. A região do gene da nucleoproteína do vírus isolado foi amplificada. O resultado da análise filogênica mostrou que o vírus isolado correspondeu ao genótipo D5. Este genótipo circula no continente da Ásia e há relatos sobre sua anterior circulação em São Paulo.

Palavras-chave. vírus do sarampo, vigilância do sarampo, genótipo do vírus do sarampo.

ABSTRACT

Owing to the efficient strategies for measles virus (MV) surveillance in São Paulo State, Brazil, no circulation of native measles virus was registered during the period from 2001 to 2007. In Sao Paulo State the imported measles cases were registered, being one in 2001, one in 2002, and in 2005 an unvaccinated 18-month-old child presenting fever and exanthema admitted to a private hospital was the target of epidemiological study. The Center of Epidemiological Surveillance found out that a brother of this child had had a similar disease one week before. The measles virus infection was confirmed at Adolfo Lutz Institute by detecting the MV-specific IgM antibody**,** by virus isolation on Vero/hSLAM cells culture**,** and by means of MV-RNA amplification on RT-PCR technique. A region of nucleoprotein gene from isolated virus was amplified. The phylogenetic analysis data showed that the isolated virus corresponded to genotype D5. This genotype circulates in the Asian continent**,** and it had circulated before in Sao Paulo State.

Key words. measles virus; measles surveillance; measles virus genotype

INTRODUCTION

In Brazil the goal of interrupting the transmission of endemic measles virus (MV) by the end of 2000 using strategies developed by the Pan American Health Organization (PAHO), which included recommendations for vaccination activities, intended to achieve high population immunity together with sensitive surveillance for suspected measles cases, including effective virological and serological surveillance[1,2]. However this strategy was not implemented in many countries of worldwide and measles outbreaks continued occurring[3,4,5].

Analysis of the variability in the nucleotide sequences of wild type Measles Virus (MV) has enabled the use of molecular epidemiologic techniques for measles surveillance. The molecular data, when used in conjunction with standard case reporting and investigation, can help to identify epidemiological links between geographically distinct cases and outbreaks as well as track importations

of measles[6] Also, approximately 5% of vaccine recipients experience mild symptoms (rash and fever) after vaccination and some of these cases could be misclassified as wild-type measles. Genetic characterization of viral isolates or RT-PCR products is the only laboratory test that can differentiate between vaccine associated cases and wild-type infection[7].

The virological surveillance carried out in the late 1980s and 1990s increased substantially the number of genotypes detected in cases outbreaks to eight clades designated from A to H including 23 genotypes recognized by the World Health Organization[8]. However, some genotypes (B1, D1, E, F, and G1) have not been detected in the last 15 years and are considered inactive[9].

In this study we investigated by serologically, isolation of the virus and the filogenetic analysis an imported case of the measles virus in Sao Paulo.

MATERIALS AND METHODS

Epidemiological history

On June 17th, 2005 during an airplane trip a sportsman who was returning home from a 14-day-long stay in the Asian continent (Maldives) after serological tests, he was diagnosed as having measles some days later. In same flight from Sao Paulo to Florianopolis the transmission occurred to a 5-year-old child[10].

Patient

On July 15th, 2005 an unvaccinated 18-month-old child was admitted to a private hospital to investigate a febrile rash. The epidemiological investigation carried out by the Center of Epidemical Surveillance uncovered that this child was infected by his 5-year-old brother who had had a similar disease one week before during an airplane trip.

Specimen collections

A blood sample was analyzed at the Adolfo Lutz Institute, state of Sao Paulo, Brazil. Measles infection was serologically confirmed as IgM-positive using IgM ELISA Test commercial Kit (Behring Diagnostics Gmb H Marburg). Peripheral blood mononuclear cells (PBMC) were purified from whole blood by using Ficol-Hypaque gradient centrifugation for virus isolation.

Virus Isolation

The specimens were added onto a Vero/hSLAM cells[11] recommended by Ronveaux et al[12] to isolate MV. The cell cultures were infected with 200ul of PBMC and maintained in DMEM plus and 2% fetal calf serum incubate at 37^0C. After two passages in cell cultures about 75% for characteristic cytopathic effect (CPE) was observed. Next, the cells were centrifuged and RNA was extracted from the cell pellet.

RT-PCR and Sequencing

RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, Calif, USA), and reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify either the 550-nucleotide (nt) coding for the COOH terminus of nucleoprotein (N)[13]. The reaction products were analyzed by using the DyeDeoxy terminator sequencing Kit in an ABI automatic 373 DNA sequencer (Applied Biosystems Ltd.,UK). Phylogenetic trees were constructed either by maximum likelihood(ML) analysis was carried out wit the PAUP 4.0 b10 (Sinauer Associate, Inc., Sunderland, MA, USA).

RESULTS

In 2005 and 2006 at the Adolfo Lutz Institute analyzed a total of 1.011 and 968 samples respectively, for measles specific antibodies by Elisa and two positive cases were confirmed in Sao Paulo. The genetic characterization was possible only one case during commercial air trips to Sao Paulo because the other child was diagnosed some days after the onset of symptoms. The results of the 18-month-old child indicated positive for measles-specific immunoglobulin (IgM) and were confirmed by positive virus isolation. The sequence coding for the carboxyl terminus of the nucleoprotein (243) nucleotides of this isolate was compared with the sequences of the GenBank reference strains (Table 1). The results indicated that this virus had identical N gene sequence and were member of genotype D5 (figure1). The sequence of Mvi/Sao Paulo.BRA/05 has been deposited in GenBank (accession number EF151191)

DISCUSSION

In Brazil measles has bend notifiable disease since 1968. With the adaptation of the Measles Elimination Plan in 1992, the national immunization campaign showed coverage of 96%, in 1995, 77% and 2000, 100%[14]. After the last epidemic in 1997, the experience was particularly instructive as a means to evaluate the surveillance system and Adolfo Lutz Institute, which is the reference state laboratory. Although Sao Paulo is the largest state in Brazil, with a population of 37 million people (IBGE 2007), the good strategies adapted for measles elimination were successful. From 2001 to 2006 no indigenous measles case was reported and only 3 imported cases two from Japan in 2001 and 2002 group D5[15], and one from Asia (2005) group D5 were registered. Measles imported-associated outbreaks continue to occur sporadically because of the persistence of endemic measles in other countries and the high volume of international trips. This fact strengthens the importance to continue measles surveillance and to investigate coverage in order to determine whether new policies are needed for the elimination of measles in the world.

Table 1. Characteristics of MV strains/isolates subjected to phylogenetic analysis

Isolation year	Genotype	Strain/isolate	Locatlity	Acc. No.
1954	A	Edm wt	USA	U01987
1990	C2	MVi/Vic.AU/3.90	Victoria, AUS	AF243460
1990	C2	Erlangen.DEU/90 "wtf "	Erlangen, DEU	X84872
1991	C2	MVi/Vic.AU/5.91	Victoria, AUS	AF243462
1992	C2	Prague243/92	Prague,CZN	Y17027
1993	D6	Stuttgart/2/93	Stuttgart, DEU	Y13825
1993	D5	Palau.BLA/93	Palau,BLA	L46758
1993	D5	Bangkok.THA/93/1	Bangkok,THA	AF079555
1994	D3	Taipeh.TWN/94	Taipeh, TAW	AJ250068
1994	D6	Sma94B	SPA	X84864
1995	D6	High Wycombe/234/95	High Wycombe, UNK	U29302
1997	D6	Novosibersk/97	Wroclaw, RUS	Y17032
1997	D3	MVi/OSaka C.JPN/17.97	Osaka, JAP	AB088149
1997	H1	MVi/Ámsterdam.net/27.97	China,JAP	AF193512
1997	C2	Funen 9/97	Funen, DEN	Y17025
1997	D6	BRA/42.97/35175	São Paulo, BRA	AF495863
1997	D6	Angra dos Reis/485/97	Angra dos Reis, BRA	AJ272480
1998	D6	Wroclaw/98	Wroclaw, POL	Y17026
1998	D5	MVs/Vic.AU/52.98	Victoria, AUS	AF243473
1998	D5	MVs/Vic.AU/51.98	Victoria, AUS	AF243472
1998	D5	MVs/Vic.AU/27.98	Victoria, AUS	AF243471
1998	D4	Amsterdam.Net/3.98	Nepal,DEU	AF193513
1999	D4	Pokhara.NEP/5.99	Pokhara, NEP	AJ250073
1999	D8	Janakpur.NEP/2.99/2	Janakpur, NEP	AJ250070
1999	D8	Janakpur.NEP/2.99/1	Janakpur, NEP	AJ250069
2001	H1	MVs/Toyota C.JPN/30.01	Toyota, JAP	AB104874
2001	D5	MVi/Sapporo.JPN/19.01	Sapporo, JAP	AB104876
2001	D5	MVi/São Paulo.BRA/01	São Paulo, BRA	AY425711
2001	D3	MVi/Okinawa.JPN/21.01	Okinawa, JAP	AB104875
2005	D5	*MVi/São Paulo.BRA/05	São Paulo, BRA	EF151191

(*) porposed genotype

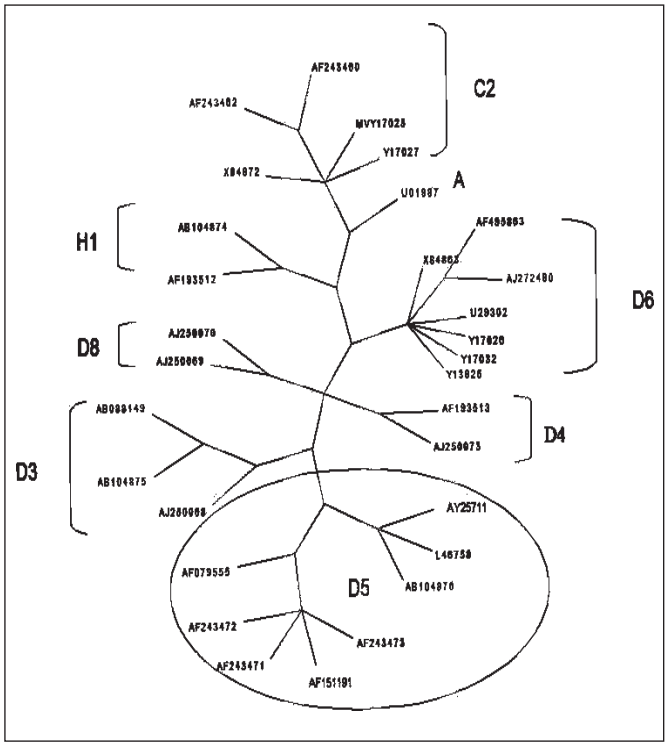

Figure 1. Phylogenetic analysis of 243nt of nucleoprotein gene of Measles Virus (MV) isolates circulating in São Paulo, Brazil. MV clustered in eigth genotypes: A, C2, D3, D4, D5, D6, D8, H1. Numbers refer to strains in Table 1.

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