Journal of Medical Virology 85:2034–2041 (2013)

Molecular Epidemiology of Rubella Viruses Involved in Congenital Rubella Infections in São Paulo, Brazil, Between 1996 and 2009

Suely Pires Curti,[1]* Cristina Adelaide Figueiredo,[1] Maria Isabel de Oliveira,[1] Joelma Queiroz Andrade,[2] Marcelo Zugaib,[2] Ana Lucia Frugis Yu,[3] Danielle Bruna Oliveira,[4] and Edison Luiz Durigon[4]

[1]*Instituto Adolfo Lutz, Núcleo de Doenças Respiratórias, São Paulo, Brazil*
[2]*Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil*
[3]*Centro de Vigilância Epidemiológica, São Paulo, Brazil*
[4]*Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, Brazil*

Rubella virus (RV) infection during the early stages of pregnancy can lead to serious birth defects, known as congenital rubella syndrome (CRS). This retrospective study was conducted between 1996 and 2009 with surveillance specimens collected from patients suspected of congenital rubella infection (CRI) and CRS. The clinical samples (nine amminiotic fluid, eight urine, eight blood, one conception product, and one placenta) were sent for viral isolation and genotyping. Twenty-seven sequences were analysed and four genotypes (1a, 1B, 1G, and 2B) were identified in São Paulo that were involved in congenital infection. To our knowledge, this study is the first report that describes genetic diversity of the circulating rubella strains involved in CRI. ***J. Med. Virol. 85: 2034–2041, 2013.***

KEY WORDS: congenital rubella infection; congenital rubella syndrome; isolation; RT-PCR; genotype

INTRODUCTION

Rubella virus (RV) is a highly infectious and teratogenic agent. Rubella virus infection during the first trimester of pregnancy may lead to fetal death or various birth defects, including deafness, cataracts, and heart disease, known as congenital rubella syndrome (CRS) [Best, 2007; Hobman and Chantler, 2007]. Live-attenuated vaccines against rubella virus have been available since the late 1960s [Hobman and Chantler, 2007]. However, globally, at least 100,000 cases of CRS occur each year [Robertson et al., 2003]. São Paulo State is the most populated Brazilian State with a population of 41 million, as of 2010. In order to control rubella and measles epidemics, the measles–rubella (MR) vaccine was introduced into the immunization program in 1992, which was preceded by a "catch-up" campaign among children 1–11 years old [Massad et al., 1995]. Despite this, however, rubella epidemics occurred in 2000/2001 and 2007/2008. The age of rubella onset increased gradually from 1999 to 2008; in the last year, the patients in most cases were 20–29 years old.

To reduce rubella transmission and prevent additional cases of CRS, Brazil began a nationwide campaign to vaccinate women of childbearing age against rubella in 2001 and another similar campaign in 2008 [Brazilian Health Department, 2010]. The World Health Organization (WHO) has recommended that countries that implement the rubella vaccine should conduct surveillance of rubella and CRS as a part of the measles surveillance system. In accordance with the established procedures of the regional measles laboratory network, public health laboratories throughout the State of São Paulo evaluate suspected cases and contacts by serologic testing, viral culture, detection of viral RNA by reverse-transcription polymerase chain reaction (PCR), and viral genotyping. In addition, the molecular epidemiology of circulating RV can be used to identify methods of transmission of the virus [WHO, 2005, 2007].

Molecular epidemiology facilitates understanding of epidemiological links during outbreaks. Genetic characterization of wild-type RV is based on sequence

*Correspondence to: Suely Pires Curti, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, CEP 01246-902, São Paulo, Brazil.
E-mail: spcurti@uol.com.br

Accepted 16 May 2013

DOI 10.1002/jmv.23675
Published online 16 July 2013 in Wiley Online Library (wileyonlinelibrary.com).

analysis of a hypervariable region of the glycoprotein E1 gene. A standard nomenclature and analysis protocol for describing wild-type RV has been established by the WHO. This nomenclature consists of two clades, 1 and 2, with nine recognized genotypes (1B, 1C, 1D, 1E, 1F, 1G, 2A, 2B, and 2C) and four provisional genotypes: 1a, 1h, 1i, and 1j. Integrated rubella surveillance that includes virological surveillance was initiated in Brazil in 1997. The epidemiological profile of the RVs involved in CRS in São Paulo is described here.

MATERIALS AND METHODS

Clinical Samples

This study was submitted to and approved by the Ethics Committee on Research with Human Beings at São Paulo University (623/CEP). This retrospective study was conducted from 1996 to 2009 with specimens collected from patients suspected of having congenital rubella infection (CRI) and CRS The Adolfo Lutz Institute, located in São Paulo is a public health laboratory and a regional reference laboratory, and receives all samples from patients with suspected infection by rubella or measles. The Institute is a member of the Program of Elimination of Rubella and CRS of the Ministry of Health of Brazil. From January 1996 to December 2009, nine amniotic fluid samples, one product of conception, one placental sample, and two blood samples were collected from patients in cases associated with primary rubella infection during pregnancy (CRI). All amniotic fluid samples were obtained at 21 weeks of gestation and 6 weeks after the onset of clinical signs of rubella infection. Samples were also collected from cases with CRS, comprising six blood and eight urine samples (Table I). Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll-Hypaque gradients and suspended in Dulbecco's minimum Eagle's essential medium (DMEM) supplemented with 2% fetal bovine serum (FBS). Urine samples were collected in sterile vessels and neutralized with sodium bicarbonate to pH 7.0. The product of conception and placenta were processed directly for PCR. All samples were stored at −80°C until tested.

Isolation and Cell Culture

Viruses were isolated by cell culture from blood and urine, and were inoculated in the Statens Serum Institut Rabbit Corneal Epithelial Cell line (SIRC; ATCC CL 60) as described previously [Figueiredo et al., 2009, 2012a]. The supernatant and cultured cells were used to determine the RV genome by PCR.

RNA Extraction and Reverse Transcription

Nucleic acid from 500 μl of amniotic fluid (AF) and 300 μl of inoculated cell culture was extracted using Tri Reagent (Molecular Research Center, Cincinnati, OH). Placental tissue was processed using the viral RNA Mini Kit (Invitrogen™, Life Technologies, Carlsbad, CA). RV RNA was detected by a previously described RT-PCR method [Bosma et al., 1995]. Both cDNA synthesis and PCR methods followed strict procedural conditions to prevent contamination, including multiple negative controls and segregated environments for pre- and post-amplification procedures. After PCR amplification, agarose gel electrophoresis and ethidium bromide staining were used to confirm the presence of a product.

Sensitivity and Specificity of Rubella Virus RT-PCR

The specificity of the RT-PCR was calculated for the 20 samples (serum, urine, and blood) by including patients who were negative for RV infection. In addition, other viruses were tested: echovirus 30; coxsackie viruses A9, B2, and B4; human coronavirus 229E; influenza virus (A and B); measles virus; parvovirus B19; herpes virus 6; respiratory syncytial virus; and mumps virus. The sensitivity of the RT-PCR method was initially assessed using a dilution series of the supernatant of an RV (RA 27/3)-infected cell culture with a known titer ($10^{6.55}$ 50% tissue culture infective doses [$TCID_{50}$] per 0.1 ml). RV RNA was extracted and amplified by RT-PCR from log dilutions (10^{-1} to 10^{-10}) of infected cell cultures and samples (urine and blood) from patients with positive serology (IgM) and which were positive for indirect immunofluorescence assay.

Genetic Characterization

An 800-nt fragment of the E1-coding region containing the 739-nt WHO-recommended sequence window (nucleotides 8,731–9,469) was amplified from samples (placenta and cerebrospinal fluid) and positive cultures using a SuperScript II One-Step RT-PCR kit (Invitrogen™, Life Technologies). An aliquot (5 μl) of RT reaction mixture was added to a PCR mix containing 10× buffer (Invitrogen™, Life Technologies), 2.5 mM $MgCl_2$, 1.25 mM of dNTP mix, and Platinum Taq DNA polymerase (Invitrogen™, Life Technologies). The forward and reverse primers used for the first reaction and nested reactions were described previously [Figueiredo et al., 2012a]. The reaction cycle parameters were 30 cycles each consisting of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min. After amplification, 5 μl of the first-round reaction mix was transferred to a new tube for the nested reaction, which was performed using the same PCR conditions as above. Products were verified on a 1.5% agarose gel stained with ethidium bromide, using 1× TAE electrophoresis running buffer. Amplified DNA fragments were purified with the PureLink PCR Purification Kit (Invitrogen) and submitted for sequencing reactions with an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Sequences obtained were analyzed

TABLE I. Summary of 26 Rubella Specimens Analyzed, São Paulo, Brazil 1996–2009

Case no.	Sequence	Sample	Suspect case	Sample RT-PCR	Cell culture virus isolation	RT-PCR culture	Genotvpe	GeneBank
1	RVi/São Paulo.BRA/27/96 CRI	Amminiotic fluid	CRI	POS	POS	POS	1a	JX524181
2	RVi/São Paulo.BRA/25/97 CRS	Blood	CRS	POS	POS	POS	1a	JX561215
3	RVi/São Paulo.BRA/01/98 CRI	Amminiotic fluid	CRI	POS	POS	POS	1a	JX524182
4	RVi/São Paulo.BRA/09/98 CRS	Amminiotic fluid	CRI	POS	POS	POS	1a	DQ788795
5	RVi/São Paulo.BRA/50/99 CRI	Amminiotic fluid	CRI	POS	POS	POS	1a	JX524183
6	RVi/São Paulo.BRA/03/00 CRI	Amminiotic fluid	CRI	POS	POS	POS	1a	JX524184
7	RVi/São Paulo.BRA/31/00 CRI	Amminiotic fluid	CRI	POS	POS	POS	1B	1X524185
8	RVi/São Paulo BRA/43/00 CRI	Amminiotic fluid	CRI	POS	POS	POS	1B	JX524186
9	RVi/São Paulo BRA/44/00 CRI	Amminiotic fluid	CRI	POS	POS	POS	1B	JX524187
10	RVi/São Paulo BRA/47/00 CRI	Amminiotic fluid	CRI	POS	POS	POS	1B	JX524188
11	RVi/São Paulo BRA/32/01 CRS	Urine	CRS	POS	POS	POS	1G	JX561216
12	RVi/São Paulo BRA/42/01 CRS	Blood	CRS	POS	POS	POS	1G	JX183246
13	RVi/São Paulo BRA/05/02 CRI	Blood	CRI	POS	POS	POS	1G	JX066727
14	RVi/São Paulo BRA/05/02 CRS	Blood	CRS	NEG	POS	POS	1G	EU220246
15	RVi/São Paulo BRA/44/02 CRI	Product of conception	CRI	POS	NP	NP	1G	EU220245
16	RVi/São Paulo BRA/20/03 CRS	Urine	CRS	POS	POS	POS	1G	JX546593
17	RVi/São Paulo BRA/20/05 CRS	Blood	CRS	POS	POS	POS	1G	JX546594
18	RVi/São Paulo BRA/49/07 CRS	Urine	CRS	POS	POS	POS	2B	JX561217
19	RVi/São Paulo BRA/08/08 CRI	Placenta	CRI	POS	NP	POS	2B	GU968196
20	RVi/São Paulo BRA/19/08 CRS	Urine	CRS	POS	POS	POS	2B	JX524610
21	RVi/São Paulo BRA/33/08 CRS	Urine	CRS	NEG	POS	POS	2B	GU968198
22	RVi/São Paulo BRA/34/08 CRS	Urine	CRS	POS	POS	POS	2B	JX524609
23	RVi/São Paulo BRA/35/08 CRS	Urine	CRS	POS	POS	POS	2B	JX524608
24	RVi/São Paulo BRA/36/08 CRS	Urine	CRS	POS	POS	POS	2B	JX546591
25	RVi/São Paulo BRA/41/08 CRS	Blood	CRS	POS	POS	POS	2B	JX546592
26	RVi/São Paulo BRA/42/09 CRS	Blood	CRS	POS	POS	POS	1a	JX546595
27	RVi/São Paulo BRA/43/09 CRI	Blood	CRI	POS	POS	POS	1G	JX546596

CRI, congenital rubella infection; CRS, congenital rubella syndrome; PW, pregnant women.
Sequence name and genotype designation based on nomenclature established by World Health Organization.

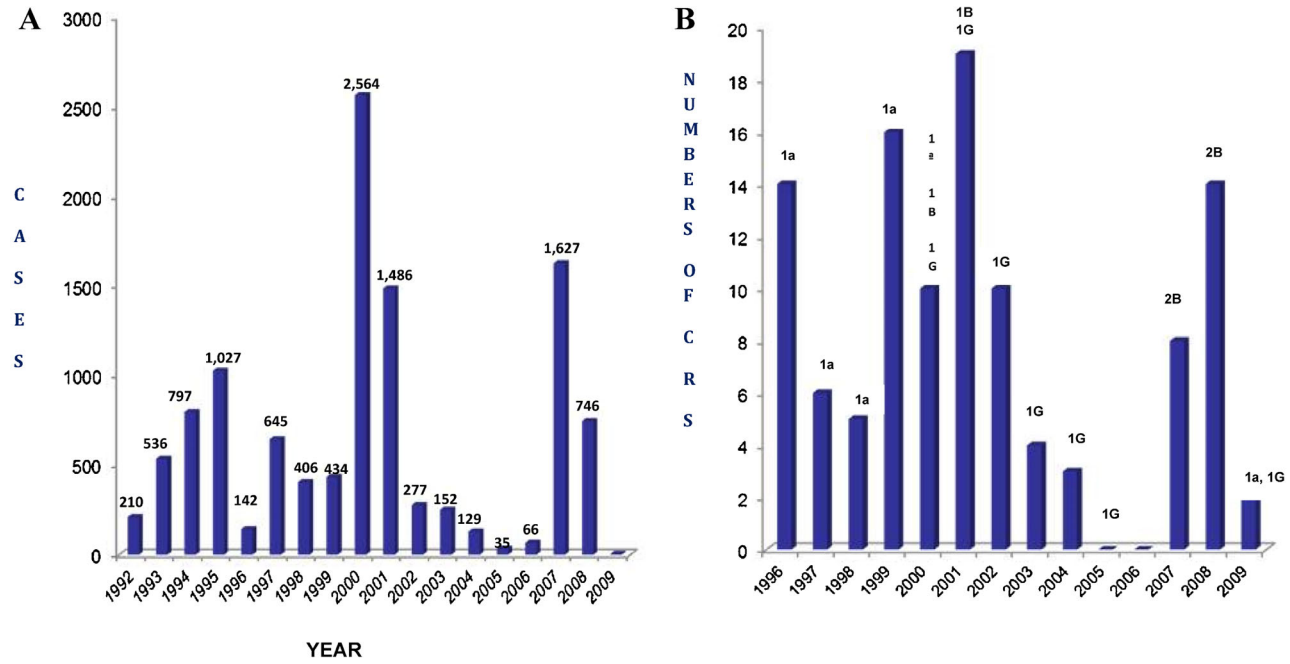

Fig. 1. **a**: Distribution of rubella cases in São Paulo, from 1992 to 2009. **b**: Numbers of CRS cases in São Paulo between 1996 and 2009 and genotypes (open bars). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

using the CLUSTAL X [Thompson et al., 1997] and BioEdit version 7.0 programs. DNA analysis software was used to compare the obtained sequences with those of the WHO reference strains. Phylogenetic analyses were performed using the DNASTAR package (DNASTAR, Madison, WI). The sequences obtained during this study are available in GenBank (Table I).

RESULTS

In this study, 13 CRI and 14 CRS cases between 1996 and 2009 were analyzed by isolation and molecular analysis of the RVs (Table I). The number of cases reported in São Paulo from 1992 to 2009 is shown in Fig. 1: rubella epidemics occurred in 2000/2001 and 2007/2008 (Fig. 1a). Two peaks of reported CRS rubella cases were observed: 1 in 2000, with 138 rubella infections in pregnant women, and the other in 2007 (70 cases; Fig. 1b). The reported rubella cases during these periods were concentrated in the age group of 20–39 years. All cases were clinically diagnosed as rubella and confirmed serologically with IgM-specific antibodies to RV. Of the samples, 92% were scored positive with a diagnostic PCR different from the PCR approach based on sequencing E1 gene fragments for genotyping. The specificity of the RT-PCR, calculated for the negative cases and viruses, was 100%. Sensitivity of viral RNA detection by this approach was 100%. All samples inoculated were positive by viral isolation, and none of the samples analyzed were negative, as determined by the culture plus RT-PCR method after the first passage.

Figure 2 shows a representative RV phylogenetic tree that was developed on the basis of the standard E1 gene window recommended by the WHO (nucleotides 8,291–9,469). These sequences, together with reference strains recommended by the WHO, were used to construct a phylogenetic tree. The 27 rubella sequences were divided into four genotypes (Figs. 1b, 2, and 3): genotype 1a (seven strains), genotype 1B (four strains), genotype 1G (eight strains), and genotype 2B (eight strains). The homology observed among all sequence isolates and the reference strains ranged from 97.8% to 98.2% for genotype 1a, from 90.0% to 93% for genotype 1G, from 97% to 98% for genotype 1B, and 98% to 99% for genotype 2B. All sequences of genotypes 1a, 1B, 1G, and 2B formed a well-supported cluster in the distance tree and grouped with the reference strains with a significant bootstrap value. RV isolates belonging to genotypes 1a, 1B, and 1G were present in São Paulo during the 2000 and 2001 outbreaks. Thereafter, in late 2007, the epidemiological conditions changed, resulting in a large outbreak of RV with a clear predominance of genotype 2B (Fig. 1b). The sequences within genotype 1a were quite similar (within-group distance: 0.9%) and clustered with sequences from Myanmar (GenBank accession numbers AY280707 and AB080199; Fig. 2). All genotype 1B isolates had 98% sequence homology with the sequence isolates from Israel (AY 968207 and AY968209) that circulated during 1975–1988. Genotype 1G obtained in this study showed a 98% similarity with viruses from Rio de Janeiro, Brazil, that circulated in 1999 (GenBank accession

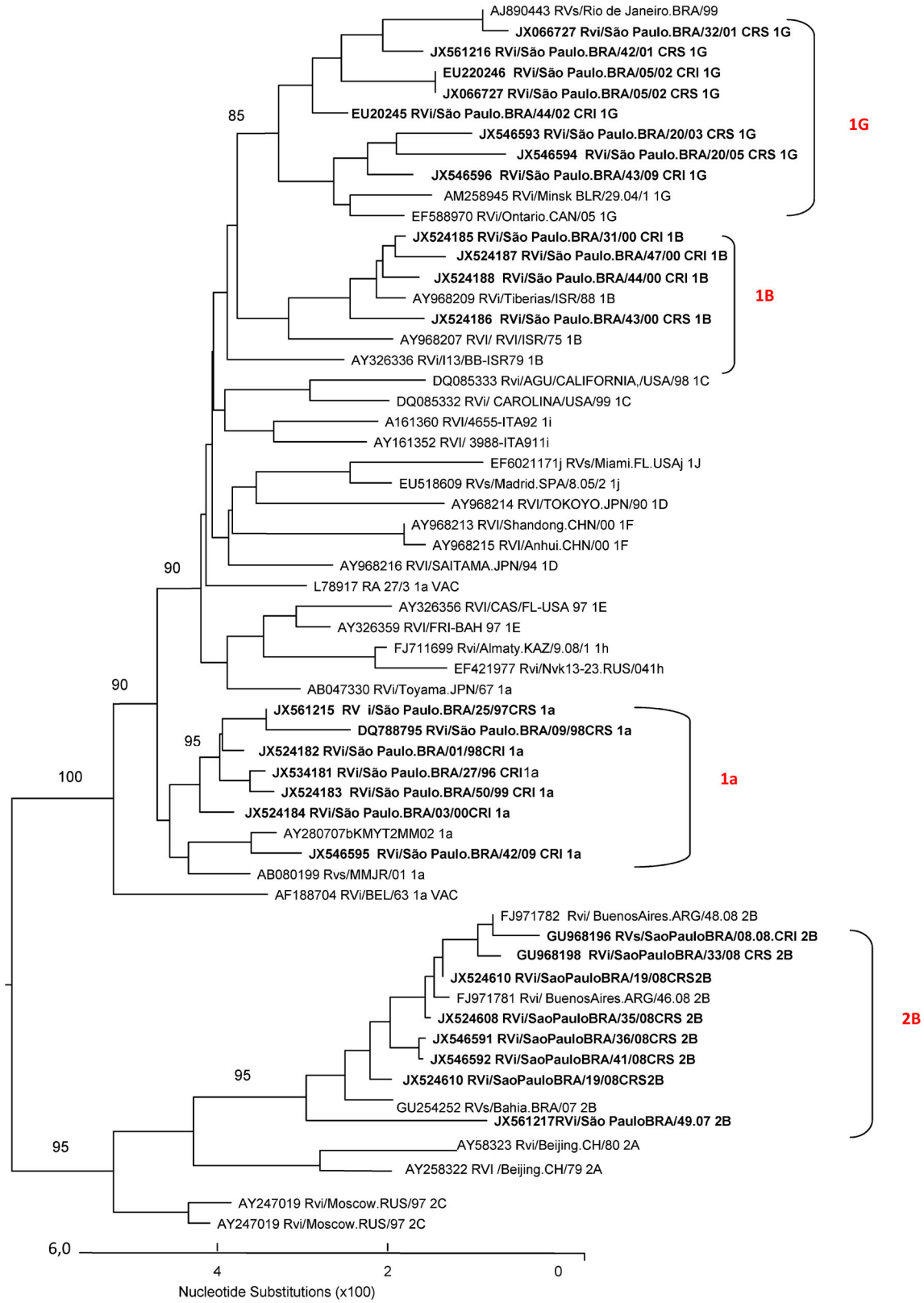

Fig. 2. Phylogenetic tree of the rubella E1 sequences in the study (bold) in comparison to reference viruses [WHO, 2005] obtained from GenBank. Reference strains and sequences from GenBank are indicated with the accession number followed by the strain name. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

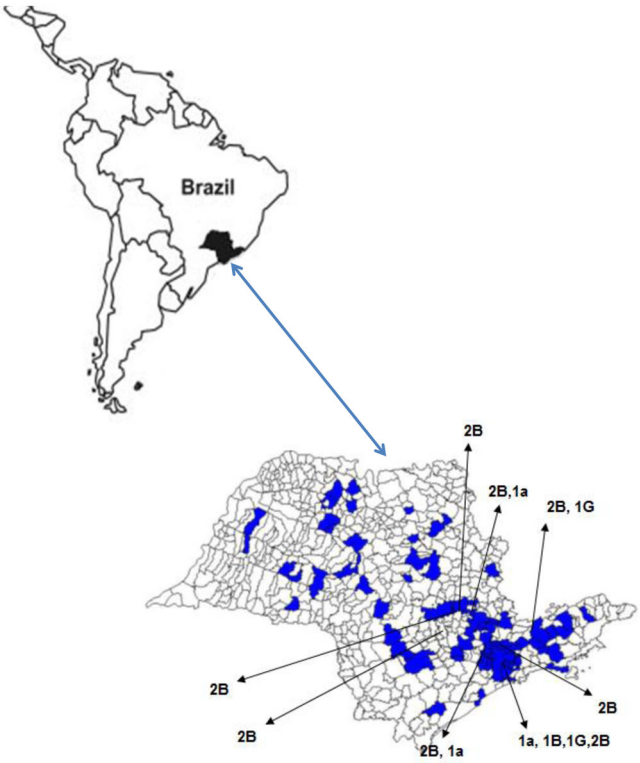

Fig. 3. Location and genotype distribution of rubella viruses isolated in São Paulo, Brazil during 1996–2009. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

number AJ890443). All genotype 2B sequences showed high similarity (99%) with viruses from Argentina (GenBank accession numbers FJ971782 and JN582036).

DISCUSSION

Rubella and CRS have been an important public health concern for Brazil. This is one of the few studies to investigate a significant number of RVs involved in congenital infections. The strains were collected from all regions of São Paulo State over a 13-year period (1996–2009), providing baseline information concerning the circulating genotypes as required for the documentation of rubella/CRS elimination. Between 1992 and 2000, the incidence of rubella among adults increased markedly, with a steady rise in the detection of CRS cases. In 2000 and 2001, two outbreaks occurred in São Paulo, with incidences reaching 7.1 per 100,000 inhabitants in 2000 and 3.8 per 100,000 inhabitants in 2001 among women aged 20–29 years (23.7 cases per 100,000 persons in this age group), with 138 confirmed rubella cases among pregnant women [CVE, 2010]. The accumulation of a large number of susceptible (unvaccinated) individuals led to the occurrence of a rubella epidemic in São Paulo in 2007; in this outbreak, most of the rubella cases involved men between 20 and 29 years of age, which differed from the demographics observed during the outbreak that occurred in 2000. A large number of cases were concentrated in the central west, south, southeast, and northeast of Brazil; there were 8,683 confirmed cases, including 161 cases of pregnant women and 17 CRS cases [Brazilian Health Department, 2010]. In São Paulo, 2,373 cases were confirmed between July 2007 and December 2008 [CVE, 2010].

During 2008, the National Plan for Measles and Rubella/CRS control launched by the Brazilian Ministry of Health strongly recommended immunization of all men and women between 20 and 39 years of age, to accelerate the elimination of rubella. Approximately 8 million men and women were vaccinated; thus, the vaccine coverage rate was 96.75% [Brazilian Health Department, 2010].

In accordance with the established procedures of the regional rubella laboratory network, public health laboratories throughout the São Paulo State evaluated the suspected cases, which included serologic testing, viral cultures, RT-PCR detection, and viral genotyping [WHO, 2005]. The RV genome was detected in 92% of the samples by RT-PCR. As the clinical diagnosis of rubella is unreliable, rapid laboratory diagnosis of rubella in pregnancy is critical. The risk of congenital infection is usually estimated by establishing the gestational age at the time of maternal infection. However, diagnosis of intrauterine infection is difficult because maternal serology is often inconclusive when infection occurs between the 13th and 20th weeks of gestation [Best, 2007]. The most direct method of diagnosing fetal infection is by PCR, and is therefore essential for early diagnosis, as shown by another study [Andrade et al., 2006].

For phylogenetic analysis, 15 sequences were obtained from viral isolates by using SIRC cell cultures and 11 were obtained directly from clinical samples (i.e., amniotic fluid, conception product, and placenta). Phylogenetic analysis of the 26 sequences obtained during this study showed that at least 4 different genotypes were involved in congenital infections: genotypes 1a, 1B, 1G, and 2B. Our study showed that there was a high incidence of rubella genotypes 1a, 1B, and 1G in pregnant women during the 2000/2001 outbreaks. The first isolation of this genotype in São Paulo was reported by Reef et al. [2002] and persisted until 2007 [Figueiredo et al., 2008; Figueiredo et al., 2011, 2012a,b]. Genotype 1a has also been responsible for the outbreaks that occurred in Myanmar and Mongolia in 2000 [Thant et al., 2006]. Recently, this genotype was found in Japan, Cambodia, and Kazakhstan [Abernathy et al., 2011], but there are no available data for comparison with the 1a strains found in São Paulo (Fig. 4). The genotype 1B found in this study was most similar to an isolate obtained in Israel more than 20 years earlier (GenBank accession number AY968209). In 2001, genotype 1B was found in France, and in 2007, in South Africa [Vauloup-Fellous et al., 2010;

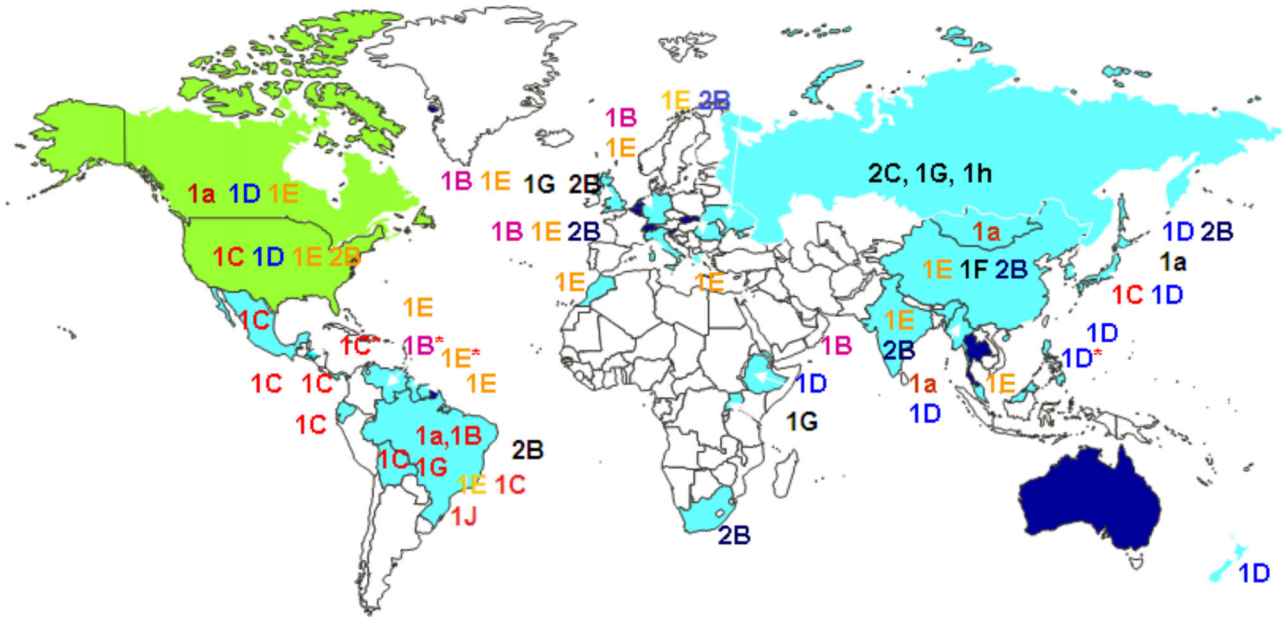

Fig. 4. Global distribution of reported rubella virus genotypes 1995–2010. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

Abernathy et al., 2011]. During the 2000–2001 outbreak, genotype 1G was also isolated and has continued to persist in Brazil [Icenogle et al., 2011]. It is important to note that genotype 1G exhibited sequence similarity to the 1G strain from Rio de Janeiro isolated during 1996–1999 [Donadio et al., 2003]. Genotype 1G is one of the prevalent genotypes of RV in Europe, Asia, and Africa (Fig. 4) [WHO, 2005, 2007; Jin and Thomas, 2007; Caidi et al., 2008; Rajasundari et al., 2008; Omer et al., 2010]. Viruses with genotypes 1G and 1B have been found in the Americas over a 7-year time span, indicating that these viruses were likely endemic [Abernathy et al., 2011]. During the 2007 outbreak, genotype 2B was the predominant rubella strain in Brazil and South America [Icenogle et al., 2011; Figueiredo et al., 2012a]. Genotype 2B viruses were previously known to circulate in Asia and Africa [WHO, 2005, 2007], and after 2006, genotype 2B was introduced to some countries in Europe [Jin and Thomas, 2007; D'Agaro et al., 2010; Vauloup-Fellous et al., 2010] and South America (Fig. 4) [Valinotto et al., 2009]. The fact that genotype 2B was not detected in the Americas before 2006 may indicate importation from Europe or Asia where this genotype is endemic. Genotype 2B found in these countries showed 99% identity with recent isolates from Japan, Argentina, and Brazil.

The Network for measles and rubella viruses surveillance reported the global distribution of RV genotypes and indicated that in Europe, genotypes 1E and 1G are predominant [WHO, 2007]. More recently, genotype 1E was isolated in cases of congenital infection from France [Vauloup-Fellous et al., 2010]. It is possible that genotype 1E has a greater propensity to cause congenital infections than other genotypes do, but it is more likely that the 1E virus was simply the most prevalent. Vauloup-Fellous et al. [2010] suggested a very wide circulation of genotype 1E, which may well be the most prevalent contemporary genotype worldwide (Fig. 4). Phylogeny showed that the French 1E sequences are interspersed with viruses from other European and American countries, possibly indicating multiple exchanges between these countries. In Brazil, consistent with national reports, genotype 1E virus not was detected [Icenogle et al., 2011]. Interestingly, the epidemiology for RV characterized in São Paulo over a 13-year period showed that genotype 1a was predominant, comprising 44% of total virus strains, followed by genotype 1G (22%), 2B (20%), and 1B (%). The detection of these genotypes coincided with rubella outbreaks in São Paulo from 2000 to 2007. In conclusion, this is the first molecular epidemiological characterization of RVs involved in CRI and CRS in Brazil. Our findings suggest that endemic transmission of RV has been interrupted. Nevertheless, rubella is still endemic in many parts of the world; therefore, it is important to maintain the surveillance system in order to document sustained absence of CRS and to maintain high vaccine coverage.

REFERENCES

Abernathy ES, Hübschen JM, Muller CP, Jin L, Brown D, Komase K, Mori Y. 2011. Status of global virologic surveillance for rubella viruses. J Infect Dis 204:S524–S532.

Andrade JQ, Bunduki V, Curti SP, Figueiredo CA, de Oliveira MI, Zugaib M. 2006. Rubella in pregnancy: Intrauterine transmission and perinatal outcome during a Brazilian epidemic. J Clin Virol 35:285–291.

Best JM. 2007. Rubella. Semin Fetal Neonatal Med 12:182–192.

Bosma TJ, Corbett KM, O'Shea S, Banatvala JE, Best JM. 1995. PCR for detection of rubella virus RNA in clinical samples. J Clin Microbiol 33:1075–1079.

Brazilian Health Department. Ministério da Saúde. Secretaria de Vigilância Epidemiológica. Available at: http://portal.saude.gov.br/portal/saude/. Accessed March 14 2010.

Caidi H, Abernathy ES, Benjouad A, Smit S, Bwogi J, Nanyunja M, El Aouad R, Icenogle J. 2008. Phylogenetic analysis of rubella viruses found in Morocco, Uganda, Cote d'Ivoire and South Africa from 2001 to 2007. J Clin Virol 42:86–90.

CVE-Center for Epidemiologic Surveillance. 2010. Available from: http://www.cve.saude.sp.gov.br/htm/Cve_dex.htm. Accessed March 25 2011.

D'Agaro P, Dal Molin G, Zamparo E, Rossi T, Micuzzo M, Busetti M, Santon D, Campello C. 2010. Epidemiological and molecular assessment of a rubella outbreak in North-Eastern Italy. J Med Virol 82:1976–1982.

Donadio FF, Siqueira MM, Vyse A, Jin L, Oliveira SA. 2003. The genomic analysis of rubella virus detected from outbreak and sporadic cases in Rio de Janeiro state, Brazil. J Clin Virol 27:205–209.

Figueiredo CA, Klautau GB, Afonso AMS, Castrignano SB, Oliveira MI, Curti SP, Squarcina GG, Narimatsu K, Rasslan Z, Lima CA, Golin V, Tadeo EF, Spagunolo FJ, Cataldo B, Durigon E. 2008. Isolation and genotype analysis of rubella virus from a case of Guillain–Barré syndrome. J Clin Virol 43:343–345.

Figueiredo CA, de Oliveira MI, Afonso AMS, Curti SP. 2009. Growth of rubella virus into SIRC (rabbit cornea cell line) using various culture media. Rev Inst Adolfo Lutz 68:145–149.

Figueiredo CA, Oliveira MI, Afonso AMS, Curti SP, Durigon EL. 2011. Rubella encephalitis in a young adult male: Isolation and genotype analysis. Infection 39:73–75.

Figueiredo CA, Oliveira MI, Curti SP, Afonso AM, Frugis Yu AL, Durigon EL. 2012a. Phylogenetic analysis of rubella virus strains during an outbreak in São Paulo, 2007–2008. J Med Virol 84:1666–1671.

Figueiredo CA, Oliveira MI, Curti SP, Afonso AM, Frugis Yu AL, Araújo J, Oliveira DB, Durigon EL. 2012b. Epidemiological and molecular characterization of rubella virus isolated in São Paulo, Brazil during 1997–2004. J Med Virol 84:1831–1838.

Hobman T, Chantler J. Rubella virus. 2007. In: Knipe DM, Howley PM, editors. Fields virology. Vol. 1. Philadelphia: Lippincott Williams & Wilkins. pp 1069–1070.

Icenogle JP, Siqueira MM, Abernathy ES, Lemos XR, Fasce RA, Torres G, Reef SE. 2011. Virologic surveillance for wild-type rubella viruses in the Americas. J Infect Dis 204:S647–S651.

Jin L, Thomas B. 2007. Application of molecular and serological assays to case based investigations of rubella and congenital rubella syndrome. J Med Virol 79:1017–1024.

Massad E, Azevedo-Neto RS, Burattini MN, Zanetta DM, Coutinho FA. 1995. Assessing the efficacy of a mixed vaccination strategy against rubella in São Paulo, Braz Int J Epidemiol 24:842–850.

Omer A, Abdel Rahim EH, Ali EE, Jin L. 2010. Primary investigation of 31 infants with suspected congenital rubella syndrome in Sudan. Clin Microbiol Infect 16:678–682.

Rajasundari TA, Sundaresan P, Vijayalakshmi P, Brown DW, Jin L. 2008. Laboratory confirmation of congenital rubella syndrome in infants: An eye hospital based investigation. J Med Virol 80: 536–546.

Reef SE, Frey TK, Theall K, Abernathy E, Burnett CL, Icenogle J, McCauley MM, Wharton M. 2002. The changing epidemiology of rubella in the 1990s: On the verge of elimination and new challenges for control and prevention. JAMA 287:464–472.

Robertson SE, Fealtherstone DA, Gacic-Dobo M, Hersh BS. 2003. Rubella and congenital rubella syndrome: Global update. Rev Panam Salud Public 14:306–315.

Thant KZ, Oo WM, Myint TT, Shwe TN, Han AM, Aye KM, Aye KT, Moe K, Thein S, Robertson SE. 2006. Active surveillance for congenital rubella syndrome in Yangon, Myanmar. Bull World Health Organ 84:12–20.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL X windows inteface: Flexible strategies for multiple sequence alignment aided by quality tools. Nucleic Acids Res 24:4876–4882.

Valinotto LE, Viegas M, Acevedo ME, Barrero PR, Mistchenko AS. 2009. Phylogenetic analysis of rubella viruses isolated in 2008 outbreak in Argentina. J Clin Virol 46:286–289.

Vauloup-Fellous C, Hübschen JM, Abernathy ES, Icenogle J, Gaidot N, Dubreuil P, Parent-du-Châtelet I, Grangeot-Keros L, Muller CP. 2010. Phylogenetic analysis of rubella viruses involved in congenital rubella infections in France between 1995 and 2009. J Clin Microbiol 48:2530–2535.

WHO. 2005. Standardization of the nomenclature for genetic characteristics of wild-type rubella viruses. Wkly Epidemiol Rec 80:126–132.

WHO. 2007. Update of standard nomenclature for wild-type rubella viruses. Wkly Epidemiol Rec 82:216–222.