Infection, Genetics and Evolution 30 (2015) 206–218

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

G1P[8] species A rotavirus over 27 years – Pre- and post-vaccination eras – in Brazil: Full genomic constellation analysis and no evidence for selection pressure by Rotarix® vaccine

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 28 November 2014
Accepted 24 December 2014
Available online 3 January 2015

Keywords:
Acute gastroenteritis
Species A rotavirus
G1P[8] genomic constellation
Rotarix® vaccine
Reassortment

ABSTRACT

Epidemiological data on species A rotavirus (RVA) infections have demonstrated the genetic diversity of strains circulating worldwide. Many G and P genotype combinations have been described over the years, varying regionally and temporally, especially in developing countries. However, the most common G and P genotype combinations identified in RVA human strains worldwide are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. RVA genotype G1P[8] strains are responsible for more than 50% of child infections worldwide and component of the two vaccines (Rotarix® [RV1] and RotaTeq® [RV5]) licensed globally. For a better understanding of the evolutionary mechanisms of this genotype in Brazil, phylogenetic analyses based on the 11 RVA genome segments (genomic constellation) from 90 G1P[8] RVA strains collected in two eras – (i) pre-vaccination with RV1 (1996–February 2006); (ii) post-vaccination (March 2006–2013) – in different Brazilian states were performed. The results showed the *Wa-like* genomic constellation of the Brazilian G1P[8] strains with a I1-R1-C1-M1-A1-N1-T1-E1-H1 specificity, except for two strains (rj14055-07 and ba19030-10) that belong to a I1-R1-C1-M1-A1-N1-**T3**-E1-H1 genomic constellation, evidencing the occurrence of reassortment (*Wa-like* × *AU-1-like*) of the NSP3 gene. Reassortment events were also demonstrated between Brazilian G1P[8] strains and the RV1 vaccine strain in some genes in vaccinated and unvaccinated children. VP7 and VP8* antigenic site analysis showed that the amino acid substitutions observed in samples collected after the introduction of RV1 in Brazil were already detected in samples collected in the 1980s and 1990s, suggesting that mass Brazilian RV1 vaccination had no impact on the diversity observed inside antigenic sites for these two proteins.

1. Introduction

Diarrheal disease (DD) represents the second leading cause of death in children ⩽five years old in the developing world (Wazny et al., 2013). Species A rotaviruses (RVA) are the main etiologic agent of DD in children in this age group worldwide (Kotloff et al., 2013), and were responsible for ~196,000 cases of severe diarrhea and deaths in developing countries in 2011 (Walker et al., 2013).

RVA is a member of the *Reoviridae* family, and the genome consists of 11 double-stranded RNA gene segments encoding six structural (VP1–4, VP6–VP7) and six nonstructural proteins (NSP1–6). A dual classification system was established for RVA based on the two genes that encode the outer capsid proteins, VP4 (P-genotype) and VP7 (G-genotype) (Estes and Greenberg, 2013). More recently, a new classification system has been proposed including all 11 genes and, to date, 27 G, 37 P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E and 11 H genotypes have been identified (Matthijnssens et al., 2008; Trojnar et al., 2013). Based on this classification, most of the human RVA detected worldwide possess one of the following genotype constellations: *Wa-like* (I1-R1-C1-M1-A1-N1-T1-E1-H1), the *DS-1-like* genotype constellation (I2-R2-C2-M2-A2-N2-T2-E2-H2) or the *AU-1-like* genotype constellation (I3-R3-C3-M3-A3-N3-T3-E3-H3), also called genotype 1, 2 and 3, respectively (Heiman et al., 2008; Matthijnssens et al., 2008; McDonald et al., 2009; Matthijnssens and Van Ranst, 2012).

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http://dx.doi.org/10.1016/j.meegid.2014.12.030
1567-1348/

Epidemiological studies of RVA infections have demonstrated the genetic diversity of strains circulating in different continents. In humans, at least six RVA G genotypes (G1–G4, G9 and more recently G12) and three P genotypes (P[8], P[4] and more recently P[6]) circulate worldwide generating a major impact on public health (Leite et al., 2008; Iturriza-Gómara et al., 2009; Bányai et al., 2012). RVA are ubiquitous, interspecies-transmitted, and accumulation of point mutations, recombination and reassortment are responsible for the huge genetic heterogeneity of these viruses. Consequently, these characteristics associated with the spreading of different RVA genotypes and genetic variants in distinct geographical regions, as well as over the seasons, may be associated with the emergence of severe DD, both spatially restricted and globally disseminated (Carvalho-Costa et al., 2011; WHO, 2013).

In Brazil, G1P[8] has been one of the most common RVA circulating genotypes during the last two decades (Santos and Hoshino, 2005; Leite et al., 2008; Carvalho-Costa et al., 2009, 2011; Rose et al., 2013). Vaccination is one of the most effective tools in reducing the consequences of RVA infections. Two vaccines, Rotarix® (RV1, GlaxoSmithKline, Brentford, Middlesex, UK) and RotaTeq® (RV5, Merck & Co., Whitehouse Station, New Jersey, USA), are licensed in several countries. Both vaccines have demonstrated broad protection against each of the most common RVA genotypes (Patton, 2012). Studies conducted in countries where RVA vaccine is provided in their national immunization programs (NIP) show the reduction of DD caused by RVA in vaccinated children (O'Ryan et al., 2011; Patel et al., 2013; Cotes-Cantillo et al., 2014). In Brazil, different studies have demonstrated the effectiveness of the RV1 vaccine in preventing hospital admission for diarrhea caused by RVA and satisfactory results against both G1P[8] and G2P[4] genotypes. These results reinforce the importance of RVA vaccination in the Brazilian NIP and the monitoring of the early emergence of unusual and novel RVA genotypes (do Carmo et al., 2011; Assis et al., 2013; de Oliveira et al., 2013; Ichihara et al., 2014; Linhares and Justino, 2014).

In a 20-year study period, Hemming and Vesikari (2013) demonstrated that mass vaccination with RV5 in Finland did not influence the genetic diversity of VP7 and VP8* proteins from G1P[8] strains. In the current study, in order to investigate whether the RV1 vaccine imposed a selective pressure on the circulation of G1P[8] in Brazil, we performed a phylogenetic analysis of the 11 genes from G1P[8] strains collected from vaccinated and unvaccinated children detected in different Brazilian regions in two different eras – pre-(1986–March 2006) and post-(March 2006–2013) RV1 introduction – in the Brazilian NIP.

2. Materials and methods

2.1. Fecal samples

RVA surveillance, which is based on a hierarchical network in which samples are provided by spontaneous demand in hospitals and health centers, monitored by the Brazilian Unified Health System (SUS), was performed between 1986 and 2013. The fecal samples were collected and sent to the central laboratory of each state and then forwarded to the Regional Rotavirus Reference Laboratory–Laboratory of Comparative and Environmental Virology (RRRL–LVCA). Forms with epidemiological, clinical and RVA vaccination status (after March 2006) accompanied each fecal sample. A total of 90 G1P[8] RVA strains was analyzed in this study and were included using the criteria as follows: at least one G1P[8] strain representative from 12 Brazilian states (11 states + Federal District) part of the reference area of our laboratory was selected, however when there was more than one G1P[8] strain in a specific state and year, only one sample was selected randomly. If eventually one outbreak was observed, more than one strain for the same state was selected. When a strain could not be completely sequenced (gene constellation), it was randomly replaced by another strain from the same state and same year. This selection strategy generated a sample size of 63 strains, which corresponds to 12% of the initial universe of the 515 G1P[8] strains. Twenty-seven strains previously studied by Rose et al. (2013) were also included in the full genome analysis. As described previously, those 27 strains represent G1P[8] strains characterized in distinct Brazilian states from 2008 to 2010, a period of low G1P[8] RVA genotype circulation (Supplementary material 1). Data concerning the RV1 coverage in the five Brazilian regions in the period between March 2006 and December 2013 are available in Supplementary material 2.

This study is part of a project that covers the diagnosis, surveillance and molecular epidemiology of viruses that cause DD approved by the Ethics Committee of Fiocruz (CEP: 311/06).

2.2. Species A rotavirus detection and G/P genotyping

RVA detection in fecal samples was carried out by enzyme immunoassay (EIA, Premier Rotaclone®, Meridian Bioscience, Inc.; Ridascreen®, R-Biopharm) following the manufacturer's recommendation and polyacrylamide gel electrophoresis (PAGE) (Pereira et al., 1983). Nucleic acids were extracted from 10% fecal suspensions by the glass powder method described by Boom et al. (1990), including modifications (Leite et al., 1996). The extracted RNA was reverse transcribed and RVA G and P genotyping was performed by the semi-nested multiplex PCR method as described previously (WHO/IVB/08.17, 2008). Fifty-seven representative G1P[8] strains from the pre-vaccination period (1986–2006) and 33 G1P[8] strains from the post-vaccination period (2007–2013) were investigated by sequence analysis of the 11 genes. Of the 33 strains detected after the year 2006, 20 belong to children vaccinated with one or two doses of RV1 (Supplementary material 1).

2.3. Eleven genes amplification and sequencing

The amplification of the 11 genome segments from selected strains were performed using a OneStep RT-PCR Kit (QIAGEN®) following the manufacturer's instructions and the following amplification conditions: (i) for VP1–2: 50 °C/30 min (min) – 95 °C/15 min – 35 cycles of 94 °C/30 seconds (s)/45 °C/30 s/72 °C/6 min, 72 °C/10 min; (ii) VP3–4: annealing temperature changed to 47 °C; (iii) for VP6, VP7, NSP1–4: 50 °C/30 minutes (min) – 95 °C/15 min – 35 cycles of 94 °C/30 seconds (s)/50 °C/30 s/72 °C/3 min, 72 °C/10 min) for NSP5: annealing temperature changed to 45 °C. Primers used to amplify the 11 gene segments are listed in Supplementary material 3. Sequencing was performed with an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit™ on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Instituto de Tecnologia em Imunobiológicos (Bio-Manguinhos / FIOCRUZ). Sequences obtained in the current study were deposited in the GenBank database under the following accession numbers: NSP1 (KM026541–KM026604), NSP2 (KM026605–KM026668), NSP3 (KM026669–KM026732), NSP4 (KM026733–KM026796), NSP5 (KM026797–KM026860), VP1 (KM026861–KM026924), VP2 (KM026925–KM026988), VP3 (KM026989–KM027052), VP4 (KM027053–KM027116), VP6 (KM027117–KM027180) and VP7 (KM027181–KM027244).

2.4. Phylogenetic analysis

Nucleotide blast analyses were performed with obtained sequences and multiple sequence alignments were carried out

using the ClustalW program (Thompson et al., 1994). Phylogenetic analyses were performed under the GTR + I model of nucleotide substitution, selected using the jModeltest program (Posada, 2008). Maximum likelihood (ML) phylogenetic trees were inferred for each one of the 11 G1P[8] gene sequences using the PhyML program (Guindon and Gascuel, 2003), in MEGA5.0 (Tamura et al., 2011). The statistical significance of the branch was assessed by bootstrap resampling analysis (1000 replicates). Deduced amino acid sequences of the 11 proteins of Brazilian G1P[8] RVA strains were compared to the RV1 strain (JX943604–JX943614) using the Bioedit v.7.2.3 software (Hall, 1999).

3. Results

RVA surveillance research was conducted for 27-year period (1986–2013) with strains obtained from children with DD (hospitalized or not) in different Brazilian regions. The G1P[8] genotype prevalence compared to other G and P genotypes ranged from 1% to 100% among Brazilian children infected with RVA in almost three decades of analysis (Fig. 1). The G1P[8] genotype presented yearly fluctuations with peaks delimited in different seasons: 1994–1995, 1997–1998, 2000–2004 and 2008–2009 (Fig. 1).

Phylogenetic trees of the 11 G1P[8] RVA genes were generated (Fig. 2). All 90 Brazilian strains analyzed belong to the *Wa-like* genotype. Eighty-eight strains showed a I1–R1–C1–M1–A1–N1–T1–E1–H1 genome constellation, while one strain detected in Rio de Janeiro in 2007, rj14055–07 (KM026726), showed a I1–R1–C1–M1–A1–N1–**T3**–E1–H1 specificity and one strain detected in Maranhão in 2010, ma19030-10 (JX683639), published previously by Rose et al. (2013), showed a Ix–R1–Cx–M1–A1–N1–**T3**–E1–H1 genome constellation (Supplementary material 1).

3.1. Sequence analysis of genes encoding structural proteins (VP)

The VP1–3 and VP6 gene analysis of the Brazilian strains showed that sequences grouped with several prototype strains collected in different countries, although some clusters show sequences exclusively collected in the same Brazilian state, as detected for strains in the state of Sergipe in 2009 (Fig. 2a–c and e). Identity values between G1P[8] Brazilian strains and RV1 vaccine ranged from 88.1% to 100% for nucleotide (nt) and 94.6% to 100% for amino acid (aa) (Supplementary material 4).

Phylogenetic analysis based on the VP8* (aa 1–247) portion of the VP4 encoding gene showed that Brazilian strains (Fig. 2d) clustered into three evolutionary lineages: (i) P[8]-1 lineage clustering the RV1 and *RV1-like* strains (se15901-08, ma19006-10 and ba19391-10); (ii) P[8]-2 lineage with strains detected in the 1980s and at the beginning of the 1990s; and (iii) P[8]-3 lineage with most Brazilian G1P[8] strains, into different P[8]-3 sublineages. The alignment of the deduced amino acid sequences showed that the potential trypsin cleavage sites at arginine 240 and 246 were both conserved in all 90 G1P[8] Brazilian strains and RV1. No change was observed on epitopes 8-2 and 8-4. The proline 68, 71, 224 and 225 residues, the cysteine residue at position 215 highly conserved among the VP8* RVA gene portion, were also maintained in all Brazilian strains and RV1 (Supplementary material 5).

Four G1 VP7 lineages (G1-I, G1-II, G1-III and G1-V) were detected in the Brazilian strains independent of the years. Identity values between G1P[8] Brazilian strains and RV1 vaccine ranged from 92.5% to 100% for nt and 93.8% to 100% for aa sequences (Supplementary material 4). G1P[8] strains detected previously in the 1990s and still circulating in Brazil were grouped into the G1-I and G1-II strains, and strains collected in different Brazilian regions in the 1980s and at the beginning of the 1990s were grouped into the G1-III and G1-V lineages (Fig. 2f).

When comparing the sequence regions defined as antigenic epitopes (7-1 and 7-2) for the VP7 protein, at least two epitopes (aa 94, 123, 148, 217) were not conserved in the Brazilian strains in comparison with the RV1 VP7 gene (Supplementary material 5).

The strain se15901-08, detected in the state of Sergipe in 2008, showed 100% nt identity with the RV1 strain for all structural genes. This strain was collected from a child who had been vaccinated with the first dose of RV1 seven days before the beginning of symptoms, evidencing a case of vaccine shedding.

3.2. Sequence analysis of genes encoding nonstructural proteins (NSP)

Similar to the core encoding VP1–3 and VP6 genes, NSP1–5 genes analysis of the Brazilian strains showed that sequences grouped with prototypes collected worldwide, although some clusters showed exclusively sequences collected in the same Brazilian state, as detected for strains in the state of Sergipe in 2009 (Fig. 2g–k). Identity values between G1P[8] Brazilian strains and RV1 vaccine ranged from 88.4% to 100% for nt and 90.7% to 100% for aa

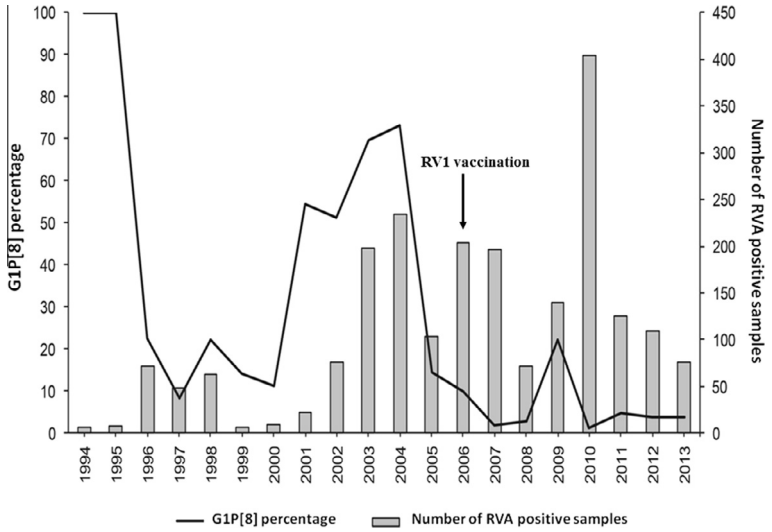


Fig. 1. Percentage of G1P[8] genotype detection on the total number of species A rotavirus (RVA) strains over the pre and post Rotarix® (RV1) vaccination eras in Brazil.

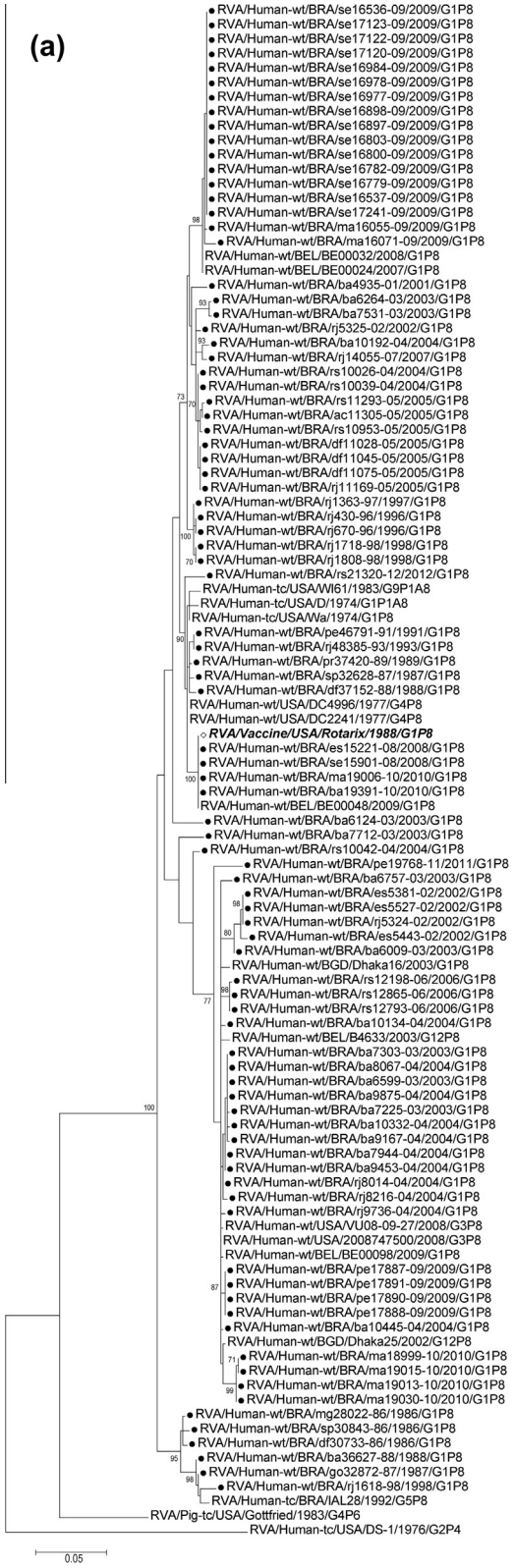


Fig. 2. Phylogenetic analysis of nucleotide sequence of the structural proteins (a) VP1 (aa 1–215), (b) VP2 (aa 1–215), (c) VP3 (aa 1–225), (d) VP8* (aa 1–287), (e) VP6 (complete cds), (f) VP7 (aa 1–324), and the non structural proteins (g) NSP1 (complete cds), (h) NSP2 (complete cds), (i) NSP3 (complete cds), (j) NSP4 (complete cds) and (k) NSP5 (complete cds) of Brazilian species A rotavirus (RVA) G1P[8] strains. The strains of the present study are indicated with ◇. The Rotarix® vaccine (RV1) strain is indicated with ●. Bootstrap values (1000 replicates) above 70% are shown at branch nodes.

Fig. 2 (*continued*)

sequences in NSP1, NSP2, NSP4 and NSP5, respectively. Three strains showed 100% aa identity with RV1 in at least one of the NSP1, NSP2, NSP4 or NSP5 genes (Supplementary material 4).

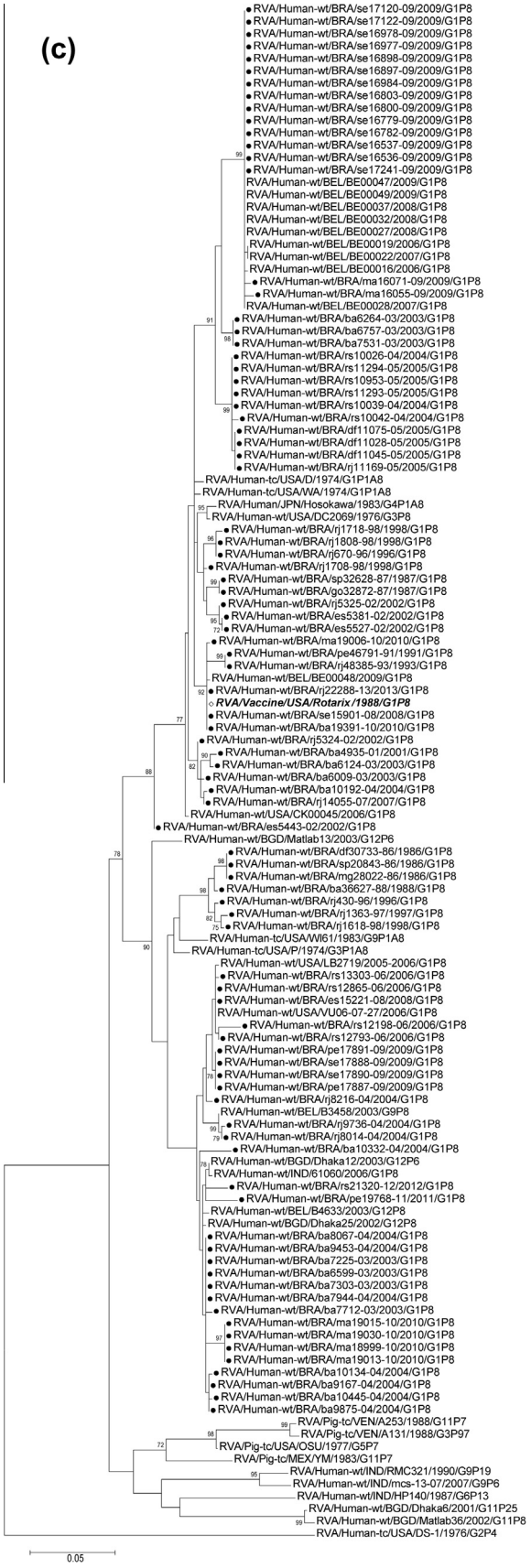


Fig. 2 (*continued*)

The NSP3 analysis showed that two strains collected in different years and in two Brazilian regions (rj14055-07 [Southern] and ba19030-10 [Northeastern]) belong to the T3 genotype. Both strains showed maximum nt identity with the prototype G3P[9] RVA (DQ490535) strain detected in Japan in 1982, AU-1 (Data not shown).

As observed for the structural genes, strain se15901-08 showed 100% nt identity with the RV1 strain for nonstructural genes. This strain was collected from a child who had been vaccinated with the first dose of RV1 seven days before the beginning of symptoms, evidencing a case of vaccine shedding.

4. Discussion

In Brazil, *Wa-like and DS-1-like* genotype combinations have been reported in cases of children infected with RVA since the decade of the 1980s. Different studies describe Brazilian prevalent combinations as: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]; however, G1P[6], G3P[6], G5P[8], G8P[8], G8P[4], G12P[8] and G12P[9] genotype combinations were detected in the country in recent decades (Mascarenhas et al., 2002; Leite et al., 2008; Tort et al., 2010; Carvalho-Costa et al., 2011; Gómez et al., 2011; Gómez et al., 2013; Gómez et al., 2014; Da Silva et al., 2011, 2013; Luchs and Timenetsky Mdo, 2014). The *Wa-like* genotype, particularly G1P[8] strains, is responsible for more than 50% of children infections worldwide and has contributed significantly to RVA DD in different age groups (Kang et al., 2009; Benati et al., 2010; Tate et al., 2010). As reported by Rose et al. (2013), G1P[8] is still detected in Brazil, even after the introduction of RV1 mass vaccination in 2006, evidencing the importance of G1P[8] full genome analysis in strains collected in different Brazilian regions for its evolutionary profile and also for the evaluation of the vaccination program. This information is also relevant in a country that has introduced an RV1 vaccine in its NIP. During the 27-year surveillance for Brazilian G1P[8], the prevalence of this genotype seems to vary annually, with changes in the relative frequencies over the years. Our findings show that Brazilian G1P[8] fluctuation shows peaks in the seasons of 1994–1995, 1997–1998, 2000–2004 and 2008–2009. Similar to our study, Giammanco et al. (2014) observed that G1P[8] presented a similar fluctuation profile over time in Parlermo, Italy, between 1985 and 2011. It is interesting to mention that despite the similar genotype prevalence in the population, Brazilian children have been vaccinated with a G1P[8] specificity vaccine (RV1) since March 2006, whereas Italy has not yet introduced an RVA vaccine in its NIP.

In the current study, one strain (se15901-08) was collected from a two-month-old child who was immunized seven days before hospitalization. Analysis of the 11 RVA genome segments showed 100% nt identity with the RV1 strain, evidencing a case of vaccine shedding. Anderson (2008) described 35–80% of healthy RV1 vaccinated children shedding the RVA vaccine strain seven days after the first dose was taken and 11–29% after the second dose. Therefore, the vaccine virus deposited by individuals into the environment may offer direct protection to the unvaccinated children; consequently the herding effect could increase and be beneficial in populations with low vaccine coverage (Rivera et al., 2011). Furthermore, studies carried out in Australia, Austria, Brazil and El Salvador reported a reduction in RVA disease and GA cases in both unvaccinated and vaccinated cohorts, suggesting that the reduction of RVA transmission by the vaccinated population could benefit the unvaccinated members of the community (Patel et al., 2012). It is not known what determines the difference observed in the vaccine efficacy in developed and developing countries,

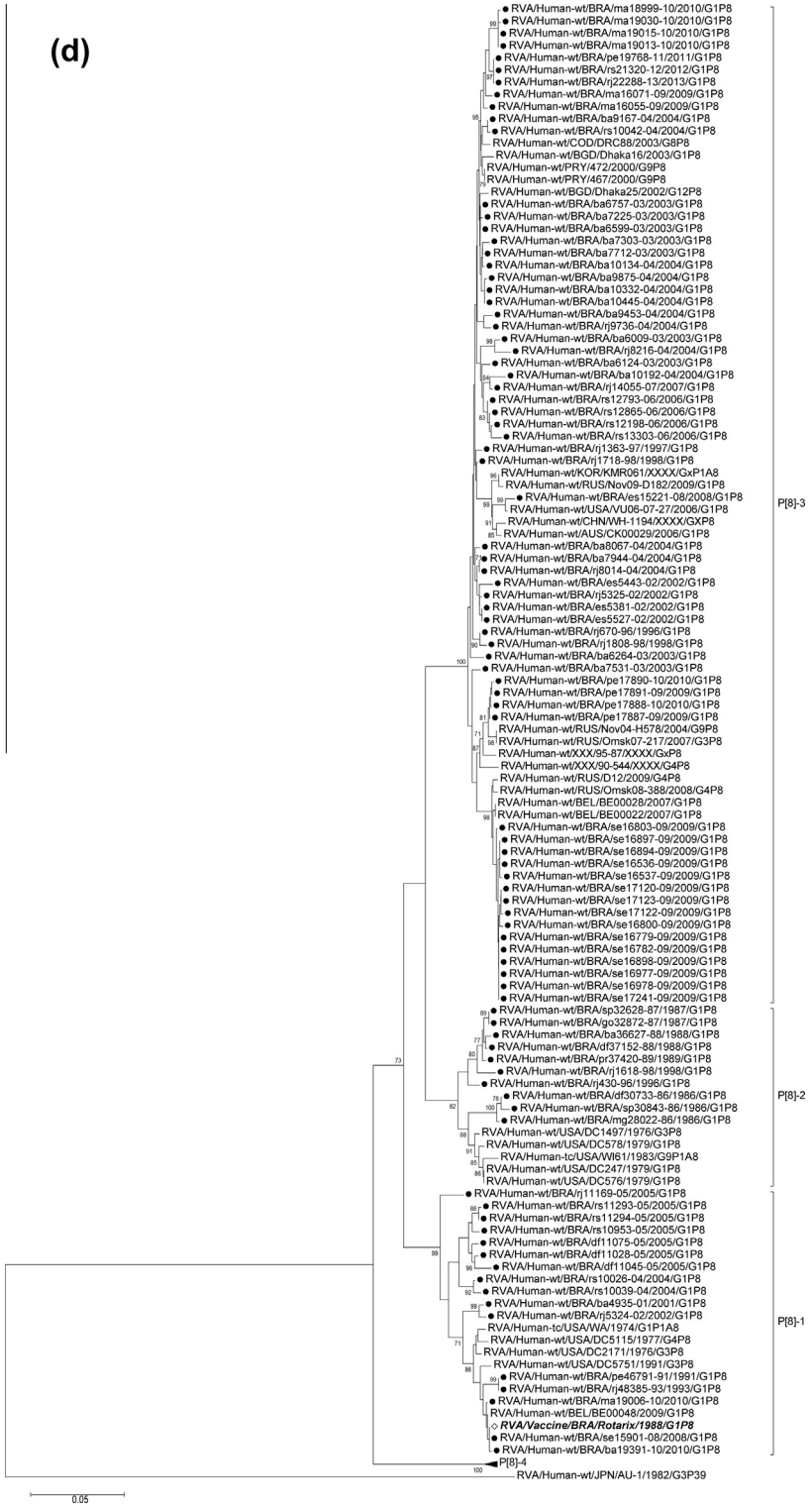
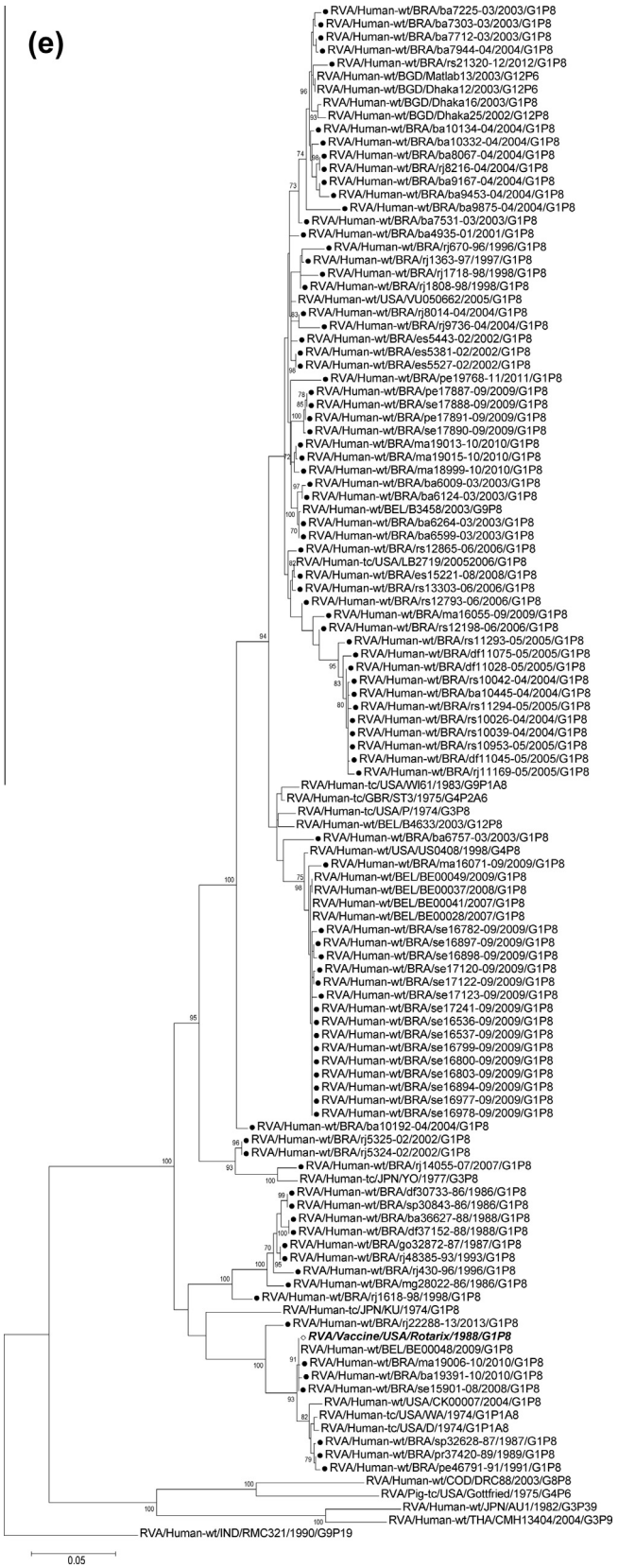


Fig. 2 (*continued*)

and a few possibilities were postulated: differences in age, nutrition status, host genetics or other clinic-epidemiological information, as recently suggested in other studies (Cherian et al., 2012; Imbert-Marcille et al., 2014; Nordgren et al., 2014; Rongsen-Chandola et al., 2014).

The study reports for the first time the 11 genome segment analysis from G1P[8] clinical strains collected in a time period of 27 years in different Brazilian regions in order to investigate how this genotype evolved in Brazil over three decades of investigation. Our results showed no evidence of selection pressure by RV1 vaccination in Brazil, as strains collected before and after 2006 (year of mass vaccination introduction in Brazil) are quite similar for all genes. It is difficult to determine the relationship between the sequences within each Brazilian region due to the reduced number



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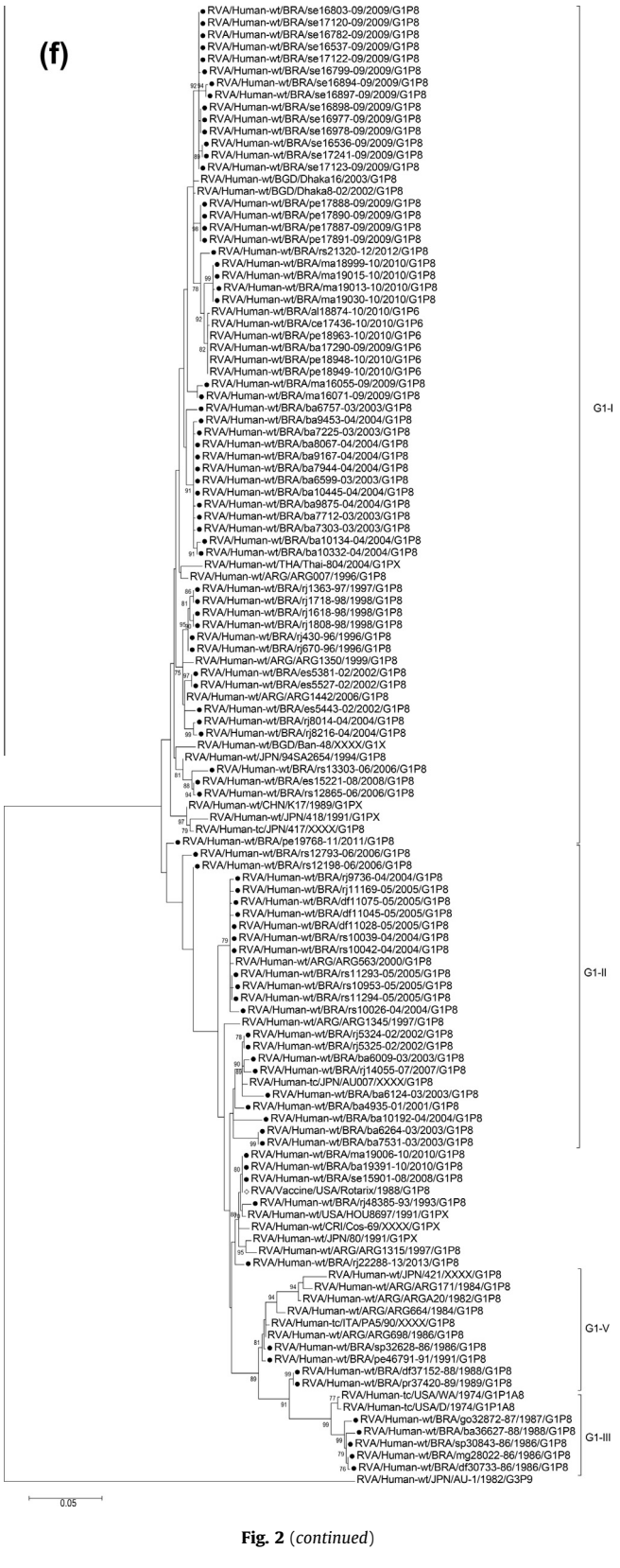

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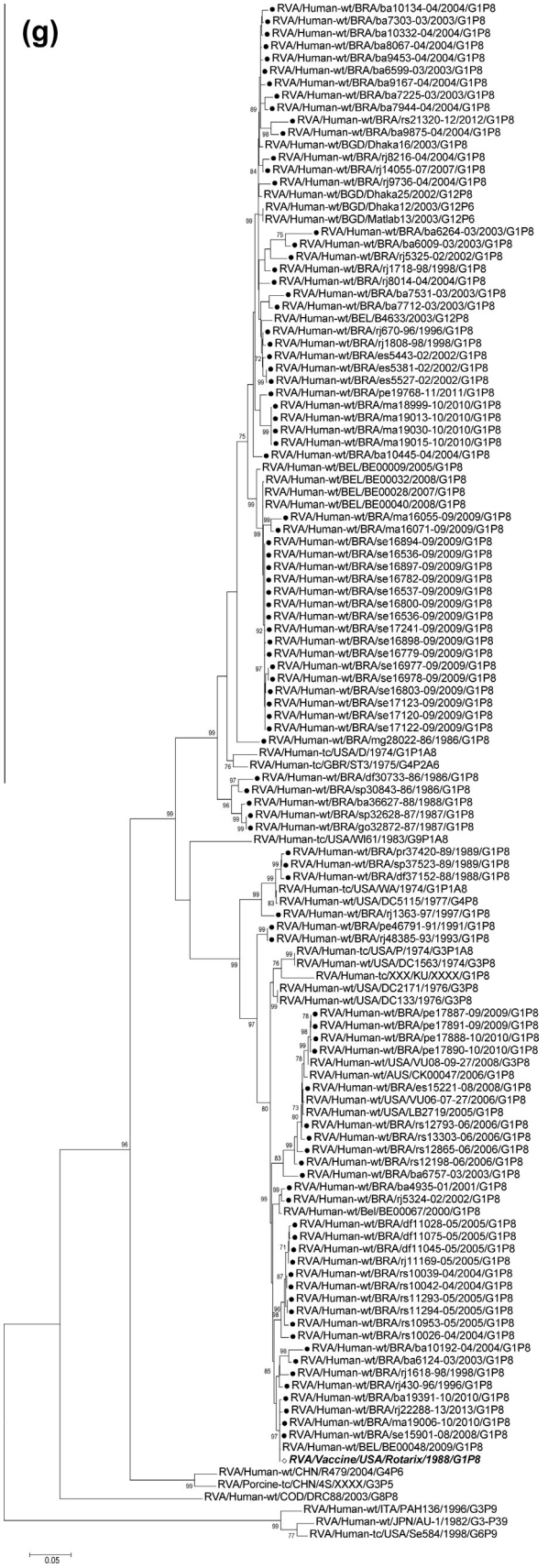
of strains for each state. Nonetheless, it is clear that multiple clusters of human *Wa-like* genotype G1P[8] RVA co-circulated and caused DD between 1986 and 2013. Rose et al. (2013) reported a reassortment event in the NSP3 gene in a strain collected from a child vaccinated with one dose in the state of Maranhão in 2010 (ma19030-10) that belongs to the T3 NSP3 genotype, related to the AU-1-like genotype constellation. In the current study, the same event was detected, for the same gene, in an unvaccinated



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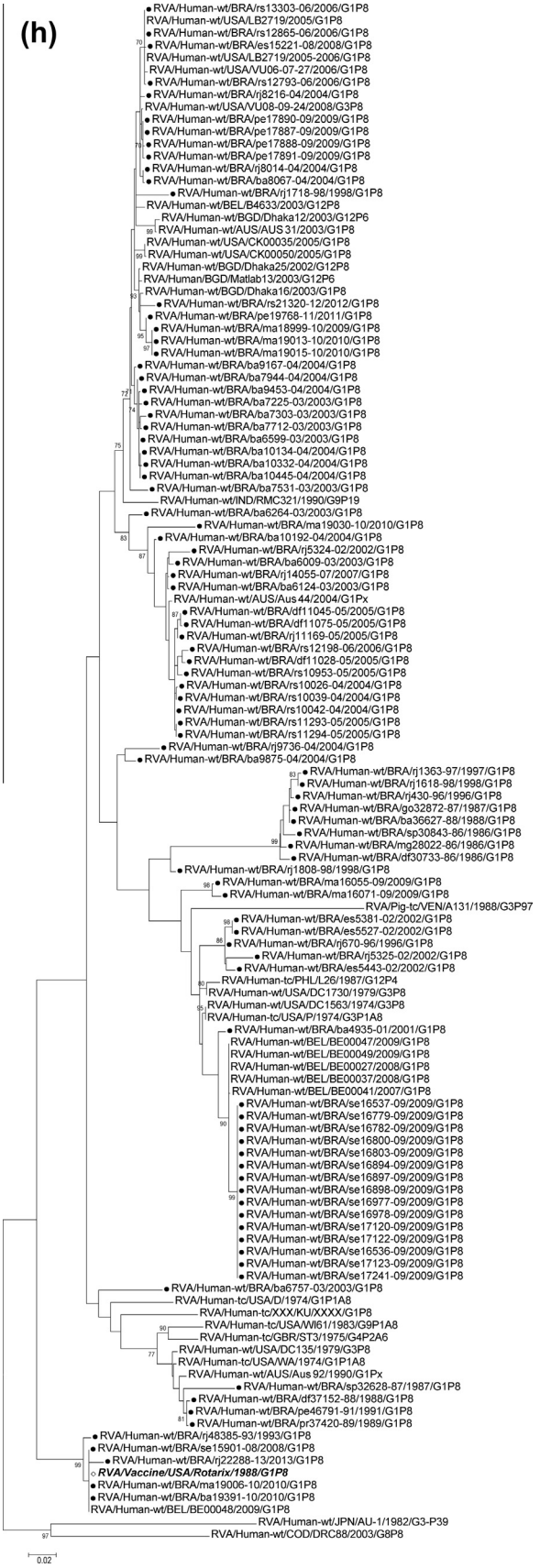

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child from Rio de Janeiro in 2007 (rj14055-07). Many studies have reported this genogrouping system to show the existence of inter-genogroup reassortments between different animal and human RVA genogroups. However, the existence and effectiveness of heterogeneous genome constellations remain unclear probably because they are caused by mechanisms that create protein sets that work better when kept together (Heiman et al., 2008).

The results obtained in the current study also showed that four Brazilian strains (es15221-08, ma19006-10, ba19391-10 and rj22288-13) were closely related to the RV1 strain, for at least one genome segment suggested the occurrence of reassortment between RV1 and wild-type strains. During a five-year surveillance study carried out in Hungary, László et al. (2012) identified 55 G1P[8] strains that were closely related but not identical to the

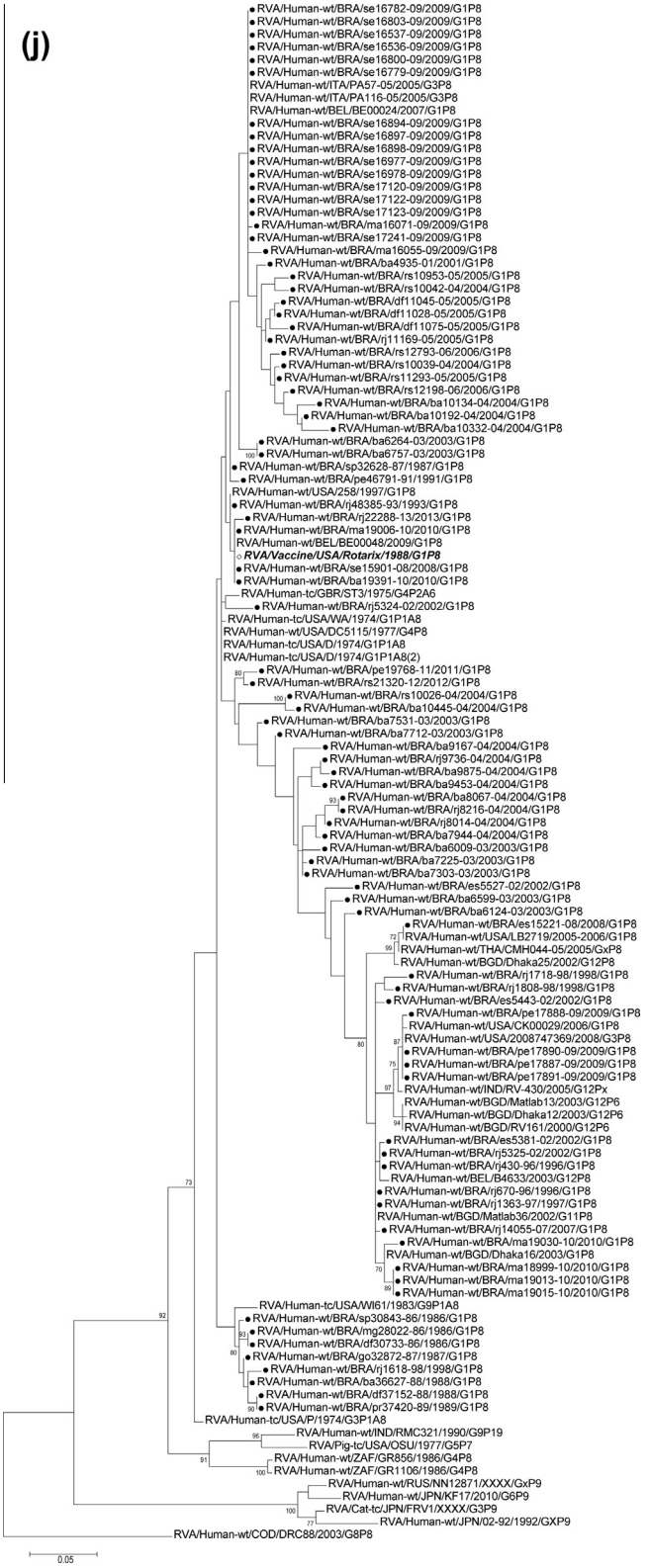

Fig. 2 (*continued*)

RV1 parental strain (strain 89-12), indicating that the vaccine strain may be circulating in the population.

The results obtained in VP1–VP3, VP6 and NSP1–NSP5 protein analysis showed that Brazilian G1P[8] strains are closely related to circulating strains belonging to genotype constellation 1

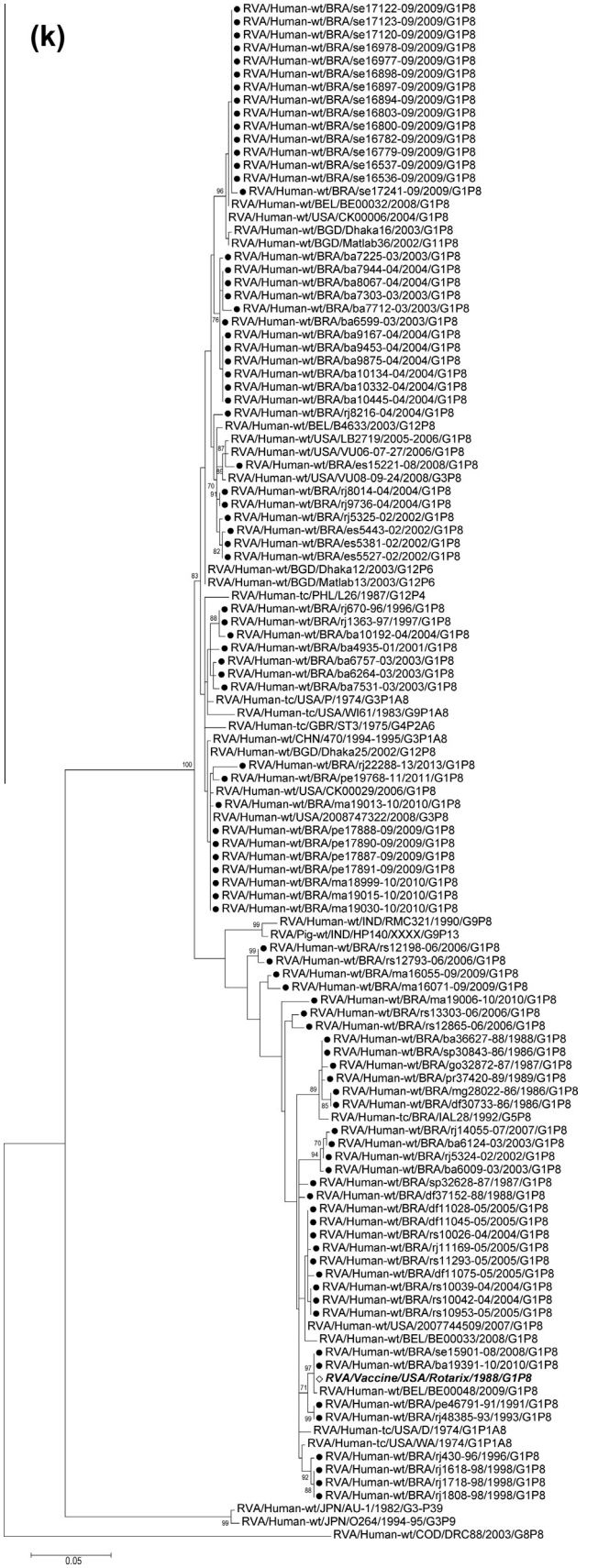

Fig. 2 (*continued*)

(*Wa-like*) collected in the same period of time worldwide, no matter the different VP7 and VP4 genotypes, corroborating previous findings in Bangladesh (Rahman et al., 2010), China (Shintani et al., 2012), India (Arora and Chitambar, 2011) and South Korea (Le et al., 2010).

Analysis of the VP8* encoding gene revealed that G1 circulated in association with different P[8] lineages in Brazil during the 27-year study: P[8]-1 lineage clustering the RV1 and RV1-like strains, P[8]-2 lineage with strains detected in the 1980s and at the beginning of the 1990s and P[8]-3 lineage with most Brazilian G1P[8] strains. P[8]-3 seems to be the only lineage currently circulating in Brazil whereas the lineage component of the RV1 vaccine is P[8]-1. da Silva et al. (2013) previously reported a great P[8]-3 Brazilian variety associated with *Wa-like* genotypes and proposed a classification into six P[8]-3 sublineages. In the present study we detected five out of six different P[8]-3 sublineages circulating in association with G1. The sublineage P[8]-3.2, observed as being exclusively associated with genotype G9 in Brazil by da Silva et al. (2013), was not detected in association with G1 in the present study, corroborating the previous results for this P[8]-3 sublineage. It is important to mention two sublineages associated exclusively with strains collected in 2009 in two states of Northeast Brazil (P[8]-3.5 grouping strains collected in Pernambuco and P[8]-3.6 with strains collected in Sergipe). In a study conducted in Finland, Hemming and Vesikari (2013) also reported the circulation of P[8]-3 in association with G1 over a period of more than 20 years. Similar results were reported by Imbert-Marcille et al. (2014) showing a wide circulation of P[8]-3 sublineages in 62 patients with diarrhea in France during 2010–2012. The P[8] VP8* protein fragment of the Brazilian strains contained differences in three (8–1, 8–2, 8–3) of the four antigenic epitopes of this fragment, corroborating previous studies that also found substitutions in VP8* antigenic epitopes of G1P[8] strains (Rahman et al., 2010; Rose et al., 2013; Hemming and Vesikari, 2013).

In the present study, analysis of the VP7 gene showed the circulation of four G1 lineages in Brazil during the last three decades. Strains collected in the 1980s and early 1990s belonged to the G1-III and G1-V lineages, despite strains collected in the late 1990s, 2000s and 2010s being grouped into G1-I and G1-II lineages, corroborating studies conducted in Argentina (Barril et al., 2013), Brazil (Maranhão et al., 2012), South Korea (Le et al., 2010) and Vietnam (Trang et al., 2012). Comparison between the VP7 genome segment and RV1 shows differences in the Brazilian G1P[8] strains. Two strains collected in the state of Rio de Janeiro from an unvaccinated child (rj14055-07) and from a child vaccinated with two doses (rj22888-13) were grouped into the G1-II lineage, with the same lineage being observed in the RV1 vaccine; however, their sequences are quite different from the RV1 strain. Various aa substitutions were detected when comparing the Brazilian strains and RV1, including changes inside antigenic sites (7-1 and 7-2); but only substitutions analysis are not sufficient to conclude if the vaccine will protect children of the infection. Only neutralization assays can confirm if these substitutions are sufficient to change the virus infectivity.

5. Conclusions

Our findings provide additional information to enable understanding of how the G1P[8] genotype has evolved in Brazil. This study suggests that the RV1 Brazilian mass vaccination does not significantly influence the G1P[8] fluctuation profile throughout the country, since most nucleotide substitutions found in samples collected after the RV1 introduction in Brazil for the 11 genes had already been observed in samples collected in the previous decades. The *Wa-like* x *Au-1-like* reassortment in two strains (NSP3 gene) was also demonstrated, along with the wild-type x RV1 vaccine strain reassortment in some vaccinated and unvaccinated children in different genes. This study, along with the study conducted by Hemming and Vesikari (2013), shows similar results for RV1 and RV5 vaccines, for the 11 genes and for VP7 and VP8*, respectively, concerning the G1P[8] genetic diversity before and after a mass RVA vaccination introduced by the NIP. Therefore, the improvement of RVA surveillance programs that include full genome sequencing analysis will contribute to improving the knowledge of some points such as how the introduction of a vaccine may affect the circulation of human or animal RVA strains, the real frequency of RVA intergenogroup reassortment events under natural conditions and the RVA strains' stability generated by such events.

Acknowledgments

This research was supported by funds from the Oswaldo Cruz Institute (IOC – Fiocruz); the National Council for Scientific and Technological Development (CNPq); the Program of Excellence in Research (PROEP – IOC/Fiocruz/CNPq); PAPES VI/Fiocruz – CNPq; Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) – project CAPES-MERCOSUL PCPP 023/2011, the General Coordination of Public Health Laboratories – Secretary of Health Surveillance (CGLAB/SVS/M of H), and the Carlos Chagas Filho Foundation for Research Support of Rio de Janeiro State (FAPERJ). The authors would like to thank Erik Vaz da Silva Leocadio from National Immunization Program, Ministry of Health, for providing information about the vaccination coverage in Brazil and the Secretary of Public Health of the different Brazilian states involved in the present study. Marcelle Figueira Marques da Silva is a PhD student of the Parasite Biology Post-Graduation Program at the Oswaldo Cruz Institute, FIOCRUZ, supported by CAPES.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.12.030.

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