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National Rotavirus Surveillance in Argentina: High incidence of G9P[8] strains and detection of G4P[6] strains with porcine characteristics

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ABSTRACT

Group A rotaviruses are the most frequently detected viral agents associated with diarrhea in infants and children worldwide. It has been estimated that every year almost 120,000 cases of diarrhea associated with rotavirus occur in children under 5 years old in Argentina. In this work, we present the rotavirus strain diversity detected during the first 2 years of the National Surveillance Network for Diarrheas implemented by the Ministry of Health in Argentina. During 2006 and 2007 a total of 464 rotavirus positive samples were G and P genotyped. The predominant genotype combination was G9P[8] (54.1%), followed by G2P[4] (26.5%) and G4P[8] (4.3%). Of note is that four samples were found possessing the G3 genotype, and two the genotype combination G4P[6]. The phylogenetic analysis of the *VP7* gene grouped the Argentinean G9 and G3 strains within the lineages currently circulating in humans worldwide, i.e. lineages III and Ia respectively; however, the sequence and phylogenetic analyses of the VP7, NSP4 and the VP8* fragment from the Argentinean G4P[6] strains suggest a porcine origin. In agreement with this, the phylogenetic tree of the *VP7* gene from G4 strains suggests the presence of at least two porcine lineages currently circulating in the Americas. In addition, the inclusion of new sequences available in public databases and the sequences reported in this work allowed us to describe new lineages and sublineages within the G4 and P[6] genotypes.

1. Introduction

Group A rotaviruses are the most frequently detected viral agents associated with diarrhea in infants and children worldwide (Parashar et al., 2006).

Based on the genetic and antigenic diversity of the two outer capsid proteins, VP7 and VP4, human and animal rotaviruses are classified into 22 G-types and 31 P-types (Matthijnssens et al., 2008; Parra et al., 2007b; Schumann et al., 2009; Solberg et al., 2009). Historically, rotaviruses bearing the combinations G1P[8], G3P[8], G4P[8] and G2P[4] were considered the most prevalent in humans worldwide; however, this picture has changed in the last decade and unusual rotavirus genotypes (such as G5, G8, G9 and G12) have been detected at high frequencies in different geographical locations. Of them, genotype G9 has been detected all around the world at high frequencies in humans since 1995, and now it is included within the five most prevalent G-types in humans (Santos and Hoshino, 2005).

The G4 strains have an overall worldwide incidence of less that 10% (Santos and Hoshino, 2005); however, this genotype has been extensively studied because its incidence increased up to ~40% in Argentina and Paraguay at the end of the 1990s and the early 2000s (Bok et al., 2002; Parra et al., 2004, 2005). The high incidences detected in these countries were attributable to the emergence of strains from two uncommon VP7 sublineages (Ib and Ic) of this genotype. Interestingly, the G4-Ic strains have been found circulating in Uruguay, Brazil, Italy, Ireland and Japan, and have been recently associated with a nation-wide outbreak of rotavirus

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in Nicaragua, with incidences of up to 80% (Arista et al., 2005; Bucardo et al., 2007; Feeney et al., 2006; Volotao et al., 2006).

Since genotype G4 is highly prevalent in the porcine population, we have previously carried out phylogenetic analyses of the *VP7* gene to compare porcine and human strains isolated worldwide, with the aim to increase our knowledge about the evolutionary dynamics of rotaviruses (Parra et al., 2008). The study revealed the presence of two human lineages and five porcine lineages and suggested a geographical distribution of the porcine strains, e.g. strains isolated in different countries of the Americas clustered together. This was also true for strains with animal characteristics isolated from humans.

In this paper, we report the rotavirus strain diversity detected during the first 2 years (i.e. 2006 and 2007) of the National Surveillance Network for Diarrheas implemented by the Ministry of Health in Argentina, and the molecular characterization of the G4P[6] strains isolated from humans that presented porcine characteristics. In addition, the inclusion of new sequences available in public databases and the sequences reported in this work allowed us to describe additional lineages and sublineages within the G4 and P[6] genotypes.

2. Materials and methods

2.1. Sample collection

A total of 2714 fecal samples were collected from January 2006 to December 2007 from infants (up to 5 years of age) with acute diarrhea, admitted as out- or inpatients to Public Hospitals from sentinel units (SU) located in the following large cities of Argentina: Buenos Aires, Córdoba, San Juan, Salta, Santa Fé and Tucumán. Since diarrhea presents a heterogeneous frequency throughout the country, being higher in the center and north and lower in the south (Gomez et al., 2002), the six SU chosen for our study were located in the center and north part of the country and were selected based on their capacity to follow a common protocol of data collection. The screening for rotavirus-positive samples was carried out using an enzyme immunoassay (IDEA™ Rotavirus, DakoCytomation, Cambridgeshire, UK) for the detection of rotavirus in human fecal samples.

2.2. Molecular characterization

The genotyping of the samples was carried out as described previously (Bok et al., 2001). Briefly, rotavirus dsRNA was extracted directly by TRIZOL® (Invitrogen, USA) or the Boom strategy when inhibitors impaired the ability to detect the genotype. The G-types were detected by amplifying the *VP7* gene using a pair of generic primers (Beg9 and End9) and then a pool of internal primers for G1, G2, G3, G4, G5 and G9, with a consensus primer 9Con1 (Bok et al., 2001; Das et al., 1994; Gouvea et al., 1990). The P-types were detected using a similar RT-PCR strategy (Gentsch et al., 1992). The amplicon of the *NSP4* gene was obtained by using consensus primers of the 5′ and 3′ end of the gene (Stupka et al., 2007). The sequencing of the *NSP4* gene and the first round amplicons of the *VP4* and *VP7* genes was carried out by the dideoxynucleotide chain terminator method on an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA). The nucleotide sequences reported in this paper were submitted to the GenBank database under the numbers: FJ712690–FJ712701 and GQ282605–GQ282614. The alignments were carried out using the BioEdit v7.0.1 (Hall, 1999). Aligned sequences were analyzed using the Modeltest program to identify the best fit-model of nucleotide substitution for phylogenetic reconstruction (Posada, 2006). The best fit-model, HKY+G, was selected under the hierarchical likelihood ratio test (hLTR). The phylogenetic relationships between strains were reconstructed by the neighbor-joining (NJ) and maximum parsimony (MP) methods using the PAUP 4.0b10 program (Swofford, 2003; PAUP 4.0b10, Sinauer, MA) and MEGA v4.0 (Tamura et al., 2007). The phylogenetic trees were also constructed using Kimura 2-parameter as a nucleotide substitution model to compare with previously published analyses.

3. Results

3.1. Genotyping

A total of 464 samples, which represented 63.1% of the total rotavirus-positive samples collected, were randomly chosen for genotyping. We found that the predominant genotype was G9P[8] (54.1%), followed by G2P[4] (26.5%) and G4P[8] (4.3%). None of the samples presented mixed infections, and in 64 (13.8%) samples the G-, the P- or even both types were not detected. Interestingly, four samples presented the G3 genotype, and two samples presented the combination G4P[6] (Table 1).

During the first year of surveillance, genotype G9P[8] was the most frequently detected in five out of the six SU. In contrast, during the second year the G9P[8] strains co-circulated at similar frequencies with G2P[4] strains (Table 2). It is worth pointing out that the four samples with genotype G3 were detected in 2007 in three different SU, which suggests the spread of this genotype throughout the country at low prevalence.

3.2. Sequence analyses

In order to confirm the results from genotyping 10 G9 strains were randomly selected for sequencing, and at least one strain from each SU was sequenced. The phylogenetic tree of the *VP7* gene grouped the Argentinean G9P[8] strains into the lineage III, closely related to the G9P[8] strains detected in Argentina during 2004 (Fig. 1a). The deduced amino acid sequence of the VP7 protein of the Argentinean G9P[8] strains showed over 98.1% of similarity between them and over 98.2% with the G9P[8] strains detected in Brazil and Paraguay, both neighboring countries of Argentina. The

Table 1
G- and P-type combinations of Argentinean rotavirus strains detected from 2006 to 2007.

G-types	P-types				
	P[4]	P[6]	P[8]	P untypeable	Total
G1	0	0	1 (0.2)	0	1 (0.2)
G2	123 (26.5)[a]	0	0	13 (2.8)	136 (29.3)
G4	0	2 (0.4)	20 (4.3)	3 (0.6)	25 (5.3)
G9	1 (0.2)	0	251 (54.1)	14 (3.0)	266 (57.3)
G3	0	0	2 (0.4)	2 (0.4)	4 (0.8)
G untypeable	2 (0.4)	0	8 (1.7)	22 (4.7)	32 (6.8)
Total	126 (27.1)	2 (0.4)	282 (60.7)	54 (11.6)	464

[a] The numbers between parentheses represent percentages.