Predominance of G9P[4] Rotavirus from Children with Acute Gastroenteritis in Northwestern Mexico

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Keywords

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Abstract

In Mexico, group A rotavirus (RVA) infections remain the most common cause of severe dehydrating diarrhea in children. This study was conducted to examine the circulating RVA strains in the northwest region of Mexico. RVA strains collected from stool samples of children were genotyped, and their partial sequences were analyzed. RT-PCR of the VP4 and VP7 genes showed the partial G9P[4] genotype in all the samples. Sequencing and phylogenetic analysis of the partial VP7 gene amplicons of 10 strains showed that they clustered in the RVA G9 lineage III, and 7 of them showed 100% identity with the reference strain LB1562, which was collected in the USA 2 years earlier. The amino acid sequences of the VP7 and VP4 antigenic regions were highly conserved between the analyzed RVA strains. Active surveillance is important for monitoring the emergence of RVA strains and their impact on cases of gastroenteritis.

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Group A rotavirus (RVA) remains the leading cause of severe diarrhea in children worldwide [1]. In Latin America, rotavirus caused an estimated 11,631 deaths in children <5 years of age in 2000, and this number declined to 2,288 in 2013, which is attributable to the introduction of vaccines [2].

RVA is a nonenveloped virus with a genome consisting of 11 segments of double-stranded RNA surrounded by a triple-layered capsid. The outermost capsid layer consists of 2 antigenic proteins: VP7 (glycoprotein) and VP4 (protease-sensitive protein). The genes coding for these proteins are the basis for the G and P dual genotyping system. To date, 27 G and 37 P genotypes have been identified, and at least 73 G/P combinations have been reported to infect humans [3, 4]. Genotypes G1–G4 and G9 comprise 88.2% of the isolates globally. Currently, the most prevalent combinations are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [5], while others have reported the G12 genotype in combination with P[6] or P[8] [6, 7]. Since the mid-1990s, there has been a global emergence of cases caused by genotype G9 strains, which were previously reported to represent 14.6% of isolations in Latin America and the Caribbean [8]. The rapid increase in RVA G9 strain cases with genetic heterogeneity has gen-

erated much interest in its origin and epidemiological significance [9]. In Mexico, few studies have reported the distribution and circulation of RVA genotypes. During the 1990s, the genotypes that were more frequently reported in the different states of Mexico were G3, G1, P[8], and P[4], and combinations thereof [10–12]. However, in recent years, genotype G9 has emerged, mostly in combination with genotype P[4] [13]. Since information of circulating genotypes is scarce in Northwestern Mexico, we report herein the genotype of strains causing diarrhea in children aged <5 years with acute gastroenteritis and diagnosed with RVA by antigen-detection immunoassay that were treated at the Children's Hospital of the State of Sonora (HIES) between February and March 2011.

In this study, 50 RVA-positive stool samples, as determined by the latex agglutination assay (Rotalex, Orion Diagnostica, Espoo, Finland), were subjected to seminested RT-PCR to determine their VP7 and VP4 (VP8*) genotypes using primers and procedures that were previously described [14–16]. Selected strains were used for sequencing, for which an 881-bp fragment (primers VP7-F and VP7-R) of the VP7 gene and an 876-bp fragment (primers Con-2 and Con-3) of the VP4 gene were first amplified. Sequencing was performed by capillary electrophoresis (Macrogen, Seoul, South Korea), using primers VP7-R and Con-3 for VP7 and VP4 (VP8*), respectively. The sequences were analyzed using ChromasPro v1.5 software, and the web-based RotaC v2.0 (<http://rotac.regatool.be>) was used to assign the genotype [17]. The sequences were deposited in GenBank (accession No. KX011580–589 for VP4, and KU244530–539 for VP7) and were compared with strains from different countries by aligning them using the CLUSTAL W program (BioEdit system version 7.2.5). A phylogenetic tree was constructed with MEGA6 software [18] using the maximum likelihood method based on the Kimura 2-parameter model (1,000 iterations for bootstrapping). The deduced amino acid sequences of VP7 and VP4 genes were obtained using MEGA6 software and were compared with sequences from other countries obtained from GenBank.

In this study, the genotype G9P[4] was detected in all the 50 samples; no mixed infections were detected. In Mexico, RVA genotyping studies in different geographical regions have identified circulating strains. For example, during 1994–1997, G1P[8] was the predominant genotype combination (40%), followed by G3P[8] (19%) and G2P[4] (16%) [11]. The G9 genotype was first identified in the USA in 1983 and remained undetectable for approximately a decade. It reemerged in the mid-1990s, and due to the increased number of countries that have re-

ported G9, it has become the fifth most prevalent genotype globally [19]. In Mexico, G9 was detected between 1998 and 2001 in combination with genotype P[6] and in cases of mixed infections of G1 + 9P[6 + 8] [20]. In 2010, the combination G9P[4] was identified in 23 states in Mexico [13, 21]. The G9P[4] genotype has also been reported in other countries in Latin-America since the 1990s [13].

Ten 641-bp sequences of the VP7 gene were analyzed and compared with reference strains obtained from GenBank. The phylogenetic tree (Fig. 1) showed that the 10 strains were grouped into a single cluster with the reference Mexican strain Mex1157 and the USA strain LB1562. Seven of the 10 isolates (HM10, HM28, HM29, HM34, HM39, HM40, and HM45) showed 100% identities with each other and the reference strains LB1562 and Mex1157, while the isolates HM13, HM46, and HM53 had 99.8% identity with the other 7 isolates. The percentage of identity between the isolates in this study and the reference strains from other American countries ranged from 97.1% (Canada) to 98.5% (Argentina and Paraguay), and they grouped into the same cluster. At least 3 distinct lineages can be recognized from the phylogenetic analysis of the VP7 gene of the G9 strains [22]. In our study, phylogenetic analysis of the 641-bp partial VP7 gene showed that all strains were located in the lineage III cluster together with other strains from American countries (LB1562, Mex1157, BethesdaDC8, US1205/USA, Py02AP57/Paraguay, Arg2366/Argentina, R143/Brazil, and RT088-09/Canada). All of these strains are distinct from lineage II, with only 87.8–88.4% identity, and from lineage I, with 88.6–89.8% identity. Our results are consistent with other reports, which have shown that G9 strains isolated in the early 1990s or later from the USA and other countries are phylogenetically more related to each other than with the first strains isolated in the 1980s (lineage I) [23–25]. This observation has generated significant interest for determining whether new G9 variants evolved from the WI61 and F45 strains isolated in the USA and Japan in 1983 and 1985, respectively, or whether they represent an independent introduction of new strains of human or animal origin [23].

Ten 422-bp sequences of the VP4 (VP8*) gene were successfully obtained and were used for phylogenetic analysis. Analysis of the VP4 (VP8*) gene showed that all strains in this study were located in a subcluster of lineage V together with the LB1562/USA and KOL-53-09/India strains (99.7% identity), which are separated from another subcluster that included strains from other parts of the world (Fig. 1). According to a study by Espinola et al. [26],

Fig. 1. Nucleotide phylogenetic analysis of VP7 and VP4 partial gene sequences detected in this study and compared to reference strains. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (1,000 iterations for bootstrapping). Phylogenetic analyses were conducted in MEGA6. Asterisks (*) indicate the strains sequenced in this study; HM34 (‡) represents the strains HM10, HM28, HM29, HM34, HM39, HM40, and HM45; HM33 (†) represents the strains HM9, HM13, HM17, HM25, HM33, HM37, HM46, HM49, HM54, and HM63. Numbers in branches indicate the percentage of iterations corresponding to each branch. The scale bars indicate the nucleotide distance.

lineage V-grouped strains have been isolated worldwide from the early 1990s through to the present.

The partial deduced amino acid sequences of the VP7 protein were compared with human G9 sequences from GenBank. The sequence analysis included the antigenic regions A (87–101), B (142–152), C (208–221), E (189–190) and F (233–242) [9]. Eight of the strains in this study had identical sequences, while strains HM46 and HM53 had 1 amino acid substitution at position 156 (A → T) and 241 (T → I), respectively (Fig. 2).

All of the deduced amino acid sequences of the VP7 gene in this study were very similar (identity rate 98.1–99.0%) to strains US1205 and R143 (used as references for lineage III) and were clearly distinguished from lineages I (strains WI61 and F45) and II (116E) by specific amino acid differences in the antigenic regions A, B, and C, which are the regions primarily responsible for determining the G serotype [27]. All strains in this study and the Mexican reference strain Mex1157 (G9P[4]) had an I at

position 78 and 108, unlike the rest of the strains used for comparison, which contained a T. All of the strains had different amino acids in positions 208, 220, and 221 from the antigenic region C and in position 242 of region F, compared to the vaccine strain Rotarix/G1P[8]/USA.

The strain Mex1157 was collected in a 2009–2010 outbreak that occurred in several states of Mexico, especially in Chiapas [13]. The strain LB1562, collected in 2010 in Long Beach, CA, USA, had a 100% identity with the partial VP7 gene of the strains isolated in Mexico during that same year [28] and with 8 strains collected during 2011 in the present report (represented by strain HM34 in Fig. 2). Hence, it is very likely that the strains detected in the present study are the same strain that was circulating during the 2009–2010 season, and that, over a 2-year period, accumulated the mutations identified in the sequences of strains HM46 and HM53.

The deduced amino acid sequences of the VP4 (VP8*) protein were compared with human P[4] sequences

Antigenic site	A										B										C										F			
	55	57	66	68	70	73	74	78	87	100	106	108	133	139	142	145	156	166	171	179	186	208	210	220	221	241	242	250	260	261				
AB180969 (WI61/USA)	I	L	A	A	S	L	D	T	A	D	F	T	L	V	M	D	A	N	T	D	A	T	T	A	S	T	T	R	I	I				
AB180970 (F45/Japan)	.	.	.	.	L	Q	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
L14072 (116E/India)	.	V	.	T	.	Q	E	.	I	G	.	.	F	.	V	N	.	.	A	N	S	.	.	T	N	.	N	.	K	L				
AF060487 (US1205/USA)	.	.	.	.	.	Q	E	.	T	.	.	.	.	.	.	.	.	.	.	.	S	I	.	T	.	.	N	K	.	.				
AF274969 (R143/Brazil)	.	.	.	.	.	Q	E	.	T	.	.	.	.	.	.	.	.	.	I	.	S	I	.	T	.	.	N	K	.	.				
KU244529 (HM34/Mexico)*‡	.	.	.	.	.	Q	E	I	T	.	.	I	.	.	.	.	.	.	.	.	S	I	.	T	.	.	N	K	.	.				
KU244538 (HM46/Mexico)*	.	.	.	.	.	Q	E	I	T	.	.	I	.	.	.	.	T	.	.	.	S	I	.	T	.	.	N	K	.	.				
KU244539 (HM53/Mexico)*	.	.	.	.	.	Q	E	I	T	.	.	I	.	.	.	.	.	.	.	.	S	I	.	T	.	I	N	K	.	.				
KC782524 (LB1562/USA)	.	.	.	.	.	Q	E	I	T	.	.	I	.	.	.	.	.	.	.	.	S	I	.	T	.	.	N	K	.	.				
JX978846 (Mex1157/Mexico)	.	.	.	.	.	Q	E	I	T	.	.	I	.	.	.	.	.	.	.	.	S	I	.	T	.	.	N	K	.	.				
HQ702259 (BethesdaDC8/USA)	.	.	.	.	.	Q	E	.	T	.	L	.	.	I	.	.	.	.	.	G	S	I	.	T	.	.	N	K	.	.				
EF179201 (Py02AP57/Paraguay)	.	.	.	.	.	Q	E	.	T	.	.	.	.	.	.	.	.	.	.	.	S	I	A	T	.	.	N	K	.	.				
GQ154532 (Arg2366/Argentina)	.	.	V	.	.	Q	E	.	T	.	.	.	.	.	.	.	.	.	.	.	S	.	.	T	.	.	N	K	.	.				
JF965010 (RT088-09/Canada)	F	.	.	.	.	Q	E	.	T	.	.	.	.	.	.	.	.	K	.	.	S	I	.	T	.	.	N	K	.	.				
JN849114 (Rotarix/G1P8/USA)	L	.	V	.	.	E	G	.	T	.	.	.	.	L	.	.	.	.	.	G	S	Q	.	E	N	.	.	K	V	.				

Fig. 2. Partial amino acid sequence alignment of the VP7 gene of selected strains in this study. The accession numbers of the strains are shown to the left. Dots indicate similarity with strain WI61(AB180969) prototype sequence at that position. Antigenic sites are indicated above the position number in bold letters. As-

terisks (*) indicate the strains detected in this study; HM34 (‡) represents the strains HM10, HM13, HM28, HM29, HM34, HM39, HM40, and HM45, which are identical in amino acid sequence.

	▼		▼	▼						▼				
	32	35	51	55	60	87	89	99	111	120	130	133	149	150
KC782522 (LB1562/USA)	N	I	G	I	T	N	D	S	H	V	I	S	S	D
KX011584 (HM33)*†	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KM008677 (KOL-53-09/India)	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KP882220 (Bang-125/Bangladesh)	.	V	.	.	.	.	.	.	.	.	.	.	.	.
KJ870695 (KisB107/Congo)	.	V	.	.	.	.	.	.	.	.	.	.	.	.
KC442895 (2008747095/USA)	.	V	.	.	.	.	.	.	.	.	.	.	.	.
JQ069668 (RT128-07/Canada)	.	V	.	.	.	.	.	.	.	.	.	.	.	.
AB118025 (DS-1/USA)	S	V	.	.	.	.	N	N	.	I	V	N	G	N
AY855067 (R291/Brazil)	.	.	.	.	I	.	.	.	.	I	.	N	G	E
M58292 (L26/Philippines)	.	.	R	.	.	.	.	.	R	I	.	N	.	N
DQ172839 (H93/Italy)	.	.	.	V	.	.	.	.	.	I	.	N	.	.
HM467941 (LB2744/USA)	.	V	.	.	.	.	.	.	.	.	.	.	.	.
JN711111 (US09Ro104/USA)	.	V	.	.	.	S	.	.	.	.	V	.	G	.
JN849113 (Rotarix G1P1A[8]/USA)	.	.	D	.	.	.	N	.	.	M	V	D	N	E

Fig. 3. Partial amino acid sequence alignment of the VP4 gene of selected strains in this study. The accession numbers of the strains are shown to the left. Dots indicate similarity with strain LB1562/USA (KC782522) sequence at that position. Antigenic sites are in-

indicated (▼) above the position number. Asterisks (*) indicate the strains detected in this study; HM33 (†) represents the strains HM9, HM13, HM17, HM25, HM33, HM37, HM46, HM49, HM54, and HM63, which are identical in amino acid sequence.

from GenBank. The sequence analysis included the antigenic regions I35R44, I55D66, and V115G123 [29]. The amino acid sequences are shown in Figure 3. All samples sequenced for this study were 100% identical to each other and to strains LB1562/USA and KOL-53-09/India. All of the sequences had different amino acid point substitutions with respect to the vaccine strain Rotarix/G1P[8]/USA, and only 1 of the substitutions (posi-

tion 120, V → M) corresponded to the antigenic region V115G123.

This is the first RVA G9P[4] genotyping report in the northwest region of Mexico. Our study is of particular importance, as the prevalence of RVA is based solely on diagnosis by antigen-detection immunoassay, and only random samples from selected states are genotyped by the Health Ministry. Hence, there is a need to increase

surveillance for the predominant genotypes to oversee changes in circulating rotaviruses, especially in the vaccinated population. Moreover, the persistence of the G9P[4] genotype in the Northwestern Mexican population may be due to the high volume of migrant farm workers from Mexico and Central America who pass through the Sonora territory on their way to the USA. We emphasize the need for continuing surveillance to assess the suitability of current rotavirus vaccines.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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