Detection of emerging rotavirus G12P[8] in Sonora, México

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Summary. – Rotavirus is the most common cause of gastroenteritis in children up to five years of age worldwide. The aim of the present study was to analyze the genotypes of rotavirus strains isolated from children with gastroenteritis, after the introduction of the rotavirus vaccine in México. Rotavirus was detected in 14/100 (14%) fecal samples from children with gastroenteritis, using a commercial test kit. The viral genome was purified from these samples and used as a template in RT-PCR amplification of the VP4 and VP7 genes, followed by gene cloning and sequencing. Among the rotavirus strains, 4/14 (28.5%) were characterized as G12P[8], 2/14 (14.3%), as G12P (not typed), and 3/14 (21.42%) as G (not typed) P[8]. Phylogenetic analysis of the VP7 gene showed that G12 genotypes clustered in lineage III. Phylogenetic analysis revealed that VP4 genotype P[8] sequences clustered in lineage V, whereas other P[8] sequences previously reported in Mexico (2005–2008) clustered in different lineages. Rotavirus genotype G12 is currently recognized as a globally emerging rotavirus. To our knowledge, this is the first report of this emerging rotavirus strain G12P[8] in México. Ongoing surveillance is recommended to monitor the distribution of rotavirus genotypes and to continually reassess the suitability of currently available rotavirus vaccines.

Keywords: rotavirus; gastroenteritis; G12P[8]

Introduction

Rotavirus gastroenteritis is a common infectious disease that affects children up to five years of age worldwide (Desselberger *et al.*, 2009). The mortality rates from diarrhea attributable to rotavirus infection are estimated to be 453,000 child deaths per year, with most of the deaths occurring in developing countries (Tate *et al.*, 2012). Rotavirus genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] are responsible for the majority of severe cases of infections worldwide and currently available rotavirus vaccines were designed based on these five common circulating genotypes (Parashar *et al.*, 2013). Despite the worldwide prevalence of rotavirus genotypes G1–G4 and G9, several studies have described unusual genotypes such as G5, G8, and G12 (Rahman *et al.*, 2007; Santos and Hoshino, 2005). The first isolate of G12 rotavirus was reported in 1987, causing diarrhea in young children. It was not reported again until 1998 in Thailand, then subsequently in 1999–2006 in United States, India, Bangladesh, Japan, Korea, United Kingdom, Belgium, Argentina, and Brazil (Rahman *et al.,* 2007). G12 is currently recognized as a globally emerging rotavirus genotype, displaying a high rate of transmission in recent years (da Silva Soares *et al.*, 2012; Delogu *et al.*, 2015; Tort *et al.,* 2015). In 2006, the Rotavirus vaccine program was implemented in México. Although the currently available vaccine has been described as safe and efficacious, the variability in rotavirus genotypes over recent years highlights the need for constant surveillance. Therefore, the present study reports genotypic analysis of rotavirus strains isolated from children with gastroenteritis in the south of Sonora, México.
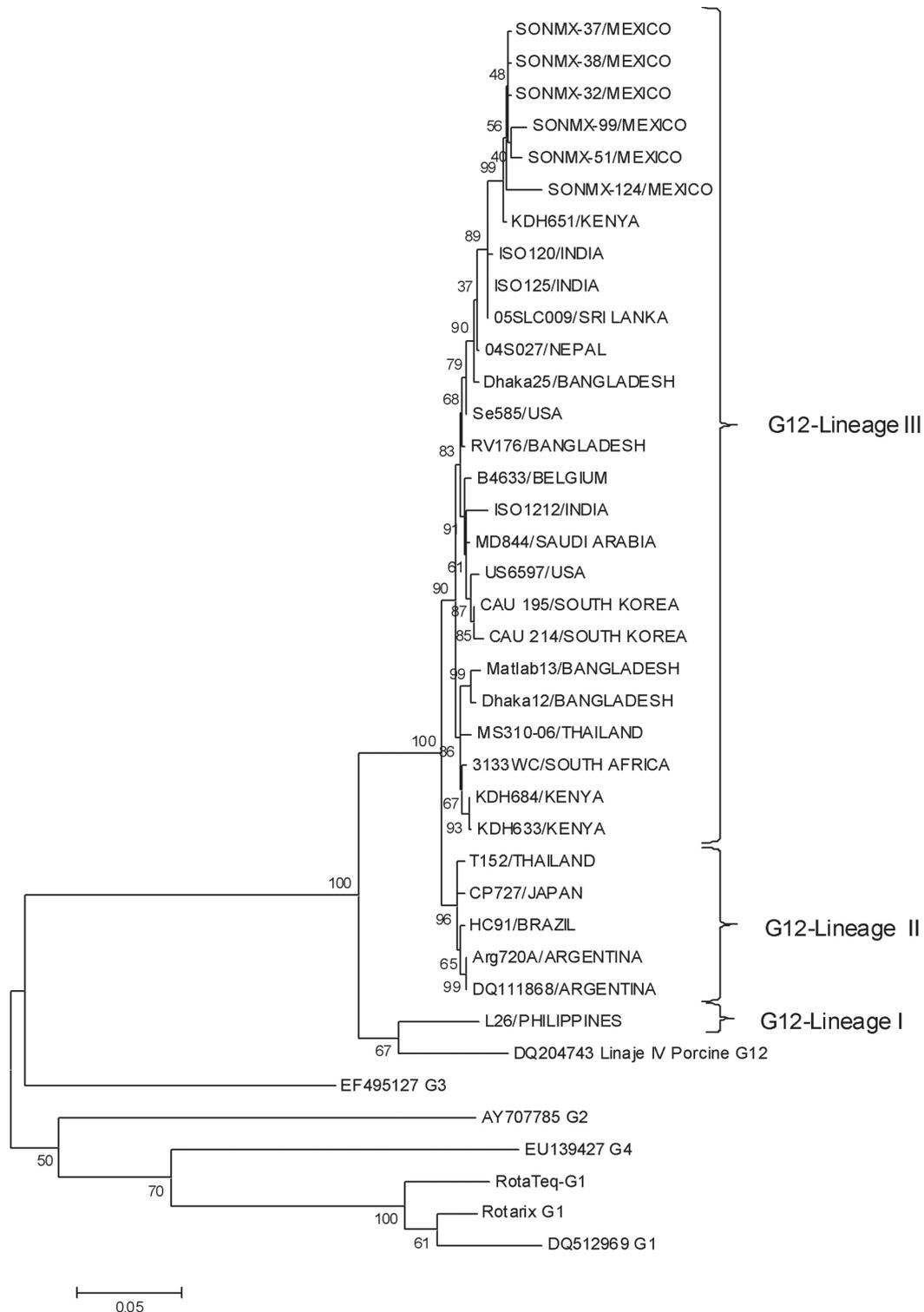
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Fig. 1

Phylogenetic analysis of the VP7 gene of genotype G12 rotavirus

Phylogenetic analysis of the nucleotide sequence of the VP7 gene of genotype G12 rotavirus strains reported in this study (SONMX-32, SONMX-37, SONMX-38, SONMX-62, SONMX-99, SONMX-124) along with reference strains downloaded from the GenBank database. The phylogenetic relationships between strains were reconstructed using the maximum likelihood method. The statistical significance of the phylogenetic trees constructed was estimated by the bootstrap method (1000 replicates).

Material and Methods

Sample collection. A total of 100 stool samples were collected from children with gastroenteritis, between August 2012 and August 2013, at Hospital del Niño y la Mujer in Cd. Obregón and Hospital General in Navojoa; both hospitals are located south of Sonora, México. To detect rotavirus positive samples, a rapid test for the detection of rotavirus antigen in stools was used according to the manufacturer's instructions (One Step Rota Adenovirus Antigen Test, Standard Diagnostics, Inc., Republic of Korea).

DNA extraction and cDNA cloning. From the rotavirus positive fecal samples, double-stranded RNA (dsRNA) was extracted using the Trizol® method (Life Technologies, USA). The purified dsRNA was used as a template for RT-PCR to amplify the VP4 (genotype P) and VP7 (genotype G) gene sequences, as previously described (Gouvea *et al.,* 1990; Gentsch *et al.,* 1992; Das *et al.,* 1994; Simmonds *et al.,* 2008). The PCR products of the VP4 and VP7 amplified genes were cloned into the pGEM-T vector (Promega, USA), following the manufacturer's instructions. Plasmids containing the VP4 or VP7 insert were purified using the Wizard SV Miniprep kit (Promega) and sequenced by the dideoxynucleotide chain termination method, using an ABI Prism BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, USA). The DNA sequence of the different clones was confirmed by sequencing of both DNA strands with the pUCM13 standard primers. The Acc. Nos for VP4 and/or VP7 sequences reported in this study are: SONMX-32 (KP119460, KP119464,), SONMX-37 (KP119461, KP119465), SONMX-38 (KT852964), SONMX-39 (KT852962), SONMX-51 (KT852965), SONMX-62 (KT852963), SONMX-99 (KP119462, KP119466), SONMX-124 (KP119463, KP119467).

Phylogenetic analysis and sequence alignment. The sequences were analyzed using MEGA 5.0 software (Tamura *et al.,* 2011) and compared with other sequences reported in the GenBank database, including the VP4 and VP7 gene sequences of the licensed vaccines Rotarix and RotaTeq. The phylogenetic relationships between strains were reconstructed using the maximum likelihood method with 1000 replicates to test phylogeny (Tamura *et al.,* 2011). The phylogenetic analysis was achieved using the partial G12 gene sequence (nt 87–1016) and the P[8] sequence (nt 142–762). The deduced amino acid (aa) sequence alignment of VP7 and VP4 was achieved using the partial sequence of the antigenic region of VP7 (aa 80–175) and VP8* (aa 45–233). The NCBI Acc. Nos for the sequences for VP7 analysis are: KDH651 (AB861961), ISO125 (EU016497), ISO120 (EU016451), 05SLC009 (AB306268), 04S027(AB263987), Dhaka25(DQ146654), Se585/USA(AJ311741), RV176 (DQ490556),B4633(DQ146643), ISO1212(EU016460), MD844 (AB269689), US6597 (FJ152121), CAU 195 (EF059916), CAU 214(EF059917),MatLab13(DQ146676),Dhaka12(DQ146665), MS310-06(AB436814),3133WC(HQ657154),KDH684(AB861972), KDH633(AB861950), CP727 (AB125852), T152 (AB071404), HC91 (AY855065), Arg720A (EU496257), ARGENTINA (DQ111868) and L26 (M58290). The NCBI Acc. Nos of the sequences VP4 (region VP8*) analysis are: KDH651(AB861959), DPRU75 (KJ752364), DPRU4620 (KJ753730), BTN-120 (AB905370), HR-15 (KJ855216), Ro4439 (JN711100), RT095-07 (JQ069655.1), UK-HLD (JX411972), VU08-09-40 (KC442932), VU08-09-39 (JF790352), R1949 (HM035525), 1670SR (KJ412735), Arg7500 (JN088445), M12-15 (KC484719), GRAVP430 (AB553325), 8340 (JN580414), BE00097(JN258920), STHY125(JX470501), MX07-623 (JX012339), MX07-588 (JX012338), MX07-577 (JX012337),

Strain	G/Lineage	7-1a											7-1b				7-2								
	aa	87	91	94	96	97	104	123	125	129	130	291	201	211	238	242	143	145	146	147	148	190	217	221	264
Rotarix	G1	T	T	N	G	E	Q	S	V	V	D	K	Q	N	N	T	K	D	Q	N	L	S	M	N	G
RotaTeq	G1	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.
L26	G12/I	N	.	T	P	D	.	D	A	.	N	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
Arg720A	G12/II	S	.	T	P	D	.	D	A	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
HC91	G12/II	S	.	T	P	D	.	D	A	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
T152	G12/II	S	.	T	P	D	.	D	A	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
CP727	G12/II	S	.	T	P	D	.	D	A	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
KDH651	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
ISO125	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
05SLC009	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
04S027	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
Dhaka25	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
B4633	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
Se585	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
3133WC	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-32	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-37	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-39	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-51	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-99	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
SONMX-124	G12/III	S	.	T	P	D	.	D	S	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.
DQ204743	G12/IV	N	.	T	P	D	.	D	A	.	.	.	.	D	.	N	Q	Q	N	S	.	.	E	A	.

Fig. 2

Amino acid sequence analysis of the VP7 gene of genotype G12 rotavirus
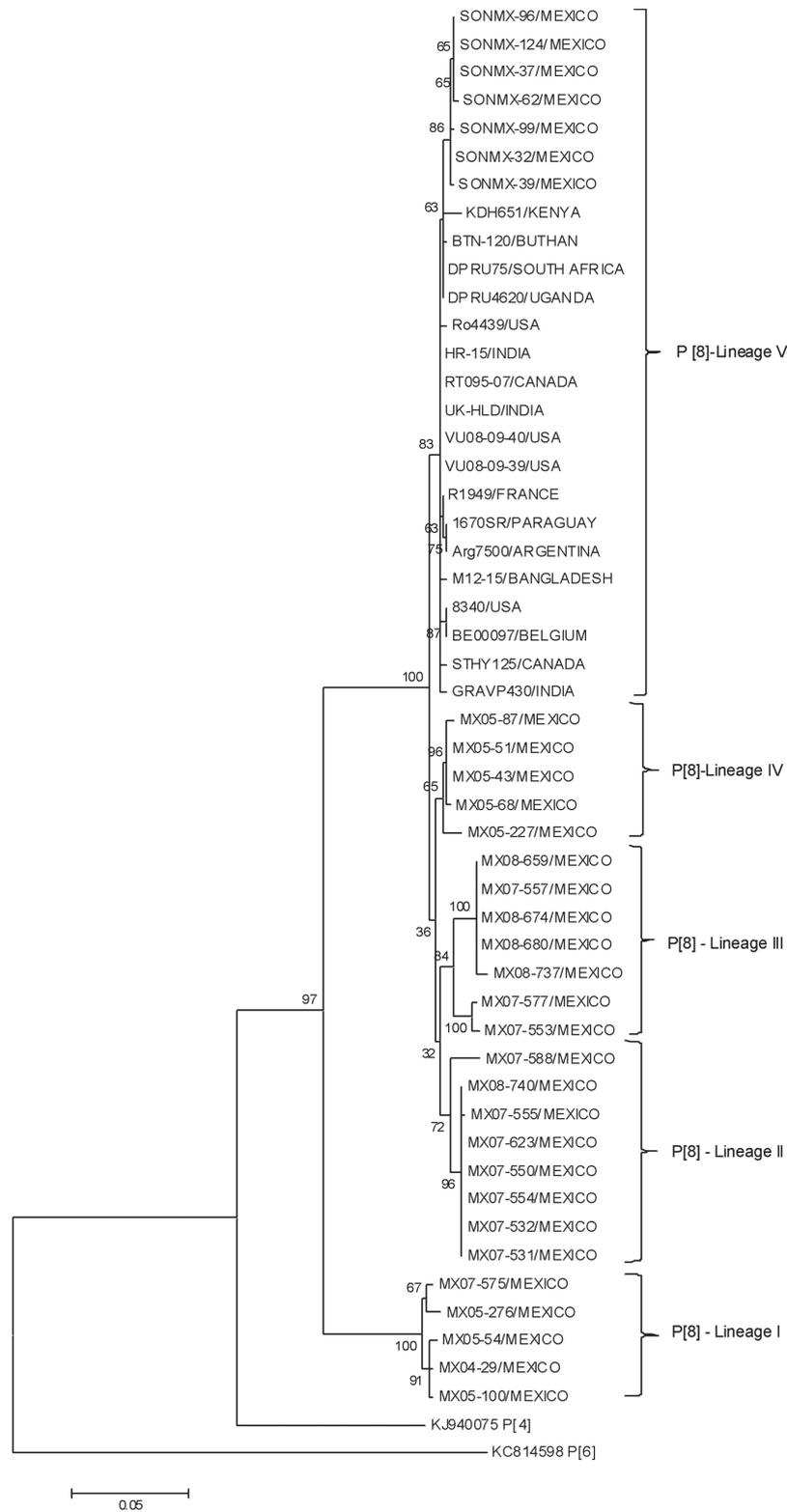Alignment of antigenic residues of the VP7 region from genotype G12 rotavirus strains.

Fig. 3

Phylogenetic analysis of the VP4 gene of genotype P[8] rotavirus

Phylogenetic analysis of the nucleotide sequence of the VP4 gene from genotype P[8] rotavirus strains reported in this study (SONMX-32, SONMX-37, SONMX-39, SONMX-62, SONMX-99, SONMX-124) along with reference strains downloaded from the GenBank database. The statistical significance of the phylogenetic trees constructed was estimated by the bootstrap method (1000 replicates).

MX07-575 (JX012336), MX07-553 (JX012335), MX07-550 (JX012334), MX05-276 (JX012333), MX05-227 (JX012332), MX05-100 (JX012331), MX05-54 (JX012330), MX08-737 (HQ585866), MX08-680 (HQ585865), MX08-674 (HQ585864), MX08-740 (FJ665391), MX08-659 (FJ665390), MX07-557 (FJ665389), MX07-555 (FJ665388), MX07-554 (FJ665387), MX07-532 (FJ665386), MX07-531 (FJ665385), MX05-68 (FJ665384), MX05-87 (FJ665383), MX05-51 (FJ665382), MX05-43 (FJ665381), MX04-29 (FJ665380), SC0028_P[6](KC814598) and KJ940075(SC19868_P[4]).

Results

Rotavirus detection and genotyping

Fourteen of the 100 samples analyzed were positive for rotavirus. All positive samples were genotyped by RT-PCR amplification of the VP4 gene (P genotype) and the VP7 gene (G genotype) (Gouvea *et al.*, 1990; Gentsch *et al.*, 1992; Das *et al.*, 1994; Simmonds *et al.*, 2008). Successful amplification was achieved for VP4 in 7/14 (50%) of the rotavirus positive samples and for VP7 in 6/14 (43%) of samples. Of the G genotypes, G12P[8] was detected in 4/14 (28.5%), G12P (not typed) was detected in 2/14 (14.3%), and G (not typed) P[8] was detected in 3/14 (21.42%) rotavirus positive samples.

Phylogenetic analysis and sequence alignment

Nucleotide sequence analysis of the VP7 gene of the G12 genotype reported in this study, revealed 98–99% identity with other G12 rotavirus strains, previously reported in Kenya (AB861961), India (EU016453), and Sri Lanka (AB306268) (Komoto *et al.*, 2014; Paul *et al.*, 2010). Phylogenetic analysis of the VP7 gene showed that G12 genotypes clustered into lineage III (Fig. 1). The alignment of antigenic residues of VP7 genotype G12 revealed some amino acid variations with respect to genotype G1 of Rotarix and RotaTeq (Fig. 2).

Moreover, the sequence of the VP8* region in the VP4 protein of rotavirus detected in this study shared 99% identity with rotavirus strains from Kenya (AB861959), Uganda (KJ753730), South Africa (KJ752364), and Bhutan (AB905370). The phylogenetic tree of the VP4 sequences revealed different lineages of P[8]. The gene sequences of P[8] reported in this study, which were isolated in 2013, belonged to lineage V, whereas other sequences reported previously in México, isolated in 2005, 2007, and 2008, clustered in different lineages (Fig. 3). Furthermore, the deduced amino acid sequence of the antigenic region of VP8* revealed the presence of sequence variations in some known antigenic epitopes in comparison with the rotavirus P[8] genotype of the vaccine strains Rotarix® and RotaTeq® (Fig. 4).

Rotavirus Strains	VP8-1											VP8-2		VP8-3									VP8-4		
	100	145	147	149	187	189	191	192	193	194	195	179	182	113	114	115	116	125	131	132	133	135	87	88	89
ROTARIX G1P[8]	D	S	S	N	S	S	A	N	L	N	N	E	R	N	P	V	D	S	S	N	D	N	N	T	N
ROTATEQ G6P[8]	.	.	.	.	N	.	.	.	.	.	D	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX04-29	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
MX05-43	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX05-51	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX05-54	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	E	.	.	.	.
MX05-68	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX05-87	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX05-227	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
MX05-276	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
MX07-531	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX07-532	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX07-550	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX07-553	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX07-554	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX07-555	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX07-557	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX07-575	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
MX07-577	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX07-588	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX07-623	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
MX08-659	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX08-674	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX08-680	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX08-737	.	G	N	.	.	N	.	.	.	.	G	.	.	D	.	.	.	N	R	.	.	D	.	.	.
MX08-740	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	N	N	R	.	.	D	.	.	.
SONMX-32	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
SONMX-37	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
SONMX-39	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
SONMX-62	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
SONMX-99	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.
SONMX-124	.	G	.	T	.	N	.	.	.	.	G	.	.	.	.	.	.	N	R	.	.	D	.	.	.

Fig. 4

Amino acid sequence analysis of the VP4 gene of genotype P[8] rotavirus
Alignment of the antigenic residues of the VP8* region from VP4 genotype P[8] rotavirus strains.

Discussion

Our results demonstrated the presence of rotavirus G12P[8] in México. To our knowledge, this is the first report of this emerging rotavirus strain in this country. Following the first report of G12 in 1987, this genotype was not reported again until 1998 (Rahman *et al.*, 2007). Over the past few years this genotype has been reported as an uncommon but emerging genotype in Asia, Europe, South America, Africa, and North America (Rahman *et al.*, 2007; Ray *et al.,* 2007; WHO, 2011; da Silva Soares *et al.*, 2012; Komoto *et al.*, 2014). Based on the increasing number of countries reporting human G12 rotavirus infection, it is possible that this rotavirus genotype will become globally dominant in the future (Patton, 2012). Phylogenetic analysis of G12 and P[8] showed an association with G12-lineage III and P[8]-lineage V. The amino acid variations in the antigenic residues of VP7 and VP4 may be indicative of strains evolving from the licensed rotavirus vaccines Rotarix® and RotaTeq®; however, more studies are required to investigate this hypothesis. This and other studies have reported the changing profile of rotavirus genotypes, with the detection of emerging and uncommon genotypes before and after the rotavirus vaccine program was introduced (Banerjee *et al.*, 2007; Le *et al.,* 2008; Bányai *et al.*, 2012; Patton, 2012; Afrad *et al.*, 2013; Wangchuk *et al.*, 2014). Although there is evidence that the implementation of rotavirus vaccines has reduced rotavirus gastroenteritis severity and associated hospitalizations (Cortes *et al.,* 2011; Leshem *et al.,* 2014), the constantly evolving genotype diversity may represent a challenge to the efficacy of currently available vaccines. Therefore, worldwide surveillance of circulating rotavirus genotypes is crucial to monitor the effectiveness of current rotavirus vaccines and to direct the development of novel vaccines in the future.

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