Emergence of Unusual Rotavirus G9p[4] and G8p[8] Strains During Post Vaccination Surveillance in Argentina, 2017-2018

Juan I Degiuseppe (jdegiuseppe@anlis.gob.ar)

Laboratorio de Gastroenteritis Virales, INEI-ANLIS “Dr. Carlos G. Malbrán” Av. Vélez Sársfield 563, Buenos Aires, Argentina https://orcid.org/0000-0002-2181-7795

Juan A Stupka

INEI - ANLIS "Dr. Carlos G. Malbrán"

Research

Keywords: diarrhea, rotavirus, surveillance, Argentina, G8P[8], G9P[4]

Posted Date: September 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-72107/v1

Version of Record: A version of this preprint was published at Infection, Genetics and Evolution on September 1st, 2021. See the published version at https://doi.org/10.1016/j.meegid.2021.104940.

Abstract

Background. In 2015, Argentina included Rotarix^{TM} monovalent vaccine for universal administration and it showed a sharp decline in all-cause and rotavirus-confirmed cases as well as an immediate predominance of the G2P[4] genotype. The aim of this study was to analyze the impact of rotavirus vaccination on disease burden and genotype distribution in our country following its introduction.

Methods. Prevalence and seasonality of laboratory-confirmed rotavirus cases data were assessed. Analyses of circulating genotypes were performed by conventional binary characterization (G and P typing). Phylogenetic study of VP7 gene was performed from emergent unusual strains.

Results. During 2017-2018, 1183 rotavirus cases (13.2%) were detected and prevalence was uniform among different age subgroups. Weekly distribution showed a raise of confirmed cases around late July and early August. In 2017 the most frequently detected genotypes were G2P[4] and G3P[8]. However, in 2018 G12P[8] genotype increased and it was detected at a high rate. Noteworthy, the detection of uncommon G9P[4] and G8P[8] strains (bearing DS-1-like genetic backbones) was observed at moderate rates.

Conclusions. Following four years of this strategy, the prevalence of rotavirus remained low in children under 5 years of age with a shift of the seasonal peak in early spring. The emergence of uncommon genotypes was due to introduction of new strains rather than to reassortment of local strains. Continuous monitoring of rotavirus burden of disease and genotype distribution provides useful evidence to evaluate existing immunization strategies and to contribute in the development of new vaccines as well.

1. Background

Since live oral rotavirus vaccines were approved and licensed, numerous countries had progressively incorporated them into their National Immunization Programs and continuous monitoring efforts to assess the impact of this strategy have been encouraged [1]. Also, surveillance studies indicate that rotavirus vaccines have significantly reduced specific hospital admissions and deaths, and overall direct and indirect burden of disease associated with acute diarrhea, as well [2]. Nonetheless, one of the questions that raise more concern is how these vaccines impact on several aspects of viral evolution such as genotype switching and the emergence of escape mutants [3].

Despite the great diversity of rotavirus strains, only six G/P associations (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8]) largely predominate in humans worldwide at present [4, 5]. However, based on the degree of overall genomic RNA homologies, it has been stated that there are two main genotypes antigenically distant comprising these frequent G/P associations: genotype 1 (or Wa-like strains, mostly bearing P[8] genotype, such as G1P[8], G3P[8], G4P[8], G9P[8] and G12P[8]), and genotype 2 (or DS-1-like strains, mostly bearing P[4] genotypes, such as G2P[4]) [6]. On the other hand, as universal vaccine introduction represents a key phenomenon of selective pressure, several countries had experienced rapid

changes in their genotype distribution with an increased diversity and the emergence of uncommon strains [7–9].

In Argentina efforts have been dedicated to sustain continuous and systematic rotavirus surveillance for extensive portrayal of the pre-vaccination scenario: overall prevalence of 25–30% with highest frequency of detection in children under 2 years, and an autumn/winter seasonality [10, 11]. Also, genotype distribution has been extensively described with co-circulation and interannual fluctuation mostly among common strains [10–13]. In 2015, Rotarix™ monovalent vaccine (human G1P[8]) was included for universal administration and its first impact study showed a sharp decrease on all-cause and rotavirus-confirmed cases and an immediate predominance of the G2P[4] genotype [14]. To assess the continuity of this intervention over time, the aim of this study is to analyze the impact of rotavirus vaccination on the burden of disease and genotype distribution in our country during the 2017–2018 period.

2. Methods

In Argentina, all-cause acute diarrhoea cases are included in the list of Mandatory Notification Events. The Argentinean Health Surveillance System (SNVS 2.0; www.sisa.msal.gov.ar*)*, which depends on the National Ministry of Health, is an online software that collects clinical and laboratory-based notifications to provide a framework for national health policies. This study represented an observational, cross-sectional, ecological analysis of laboratory confirmed rotavirus cases in children under 5 years of age, reported to the SNVS 2.0 in Argentina during 2017–2018.

2.1 Rotavirus laboratory confirmed cases

Data were extracted from the Viral Diarrhea Notification Module of the SNVS 2.0. Briefly, hospital laboratories distributed nationwide upload a weekly condensed report of the total number of rotavirus tests performed and the number of positives by either ELISA or immunochromatography, classified by age group. To ensure constant coverage, we used data from laboratories that had been reporting for at least 44 out of the 52 epidemiological weeks (EWs, ~ 85%) per year to the National Reference Laboratory during the studied period. Seasonal peak was defined as the three consecutive week period with the highest number of rotavirus cases. The proportion of positive tests was calculated from positive and negative rotavirus cases.

2.2 Rotavirus circulating genotypes

Analyses of circulating genotypes were performed on the rotavirus-positive stool specimens that hospital laboratories were able to submit to the National Reference Laboratory according to rotavirus surveillance protocols. Conventional binary characterization of the outermost capsid genes (VP7 and VP4 for G and P typing, respectively) used a hemi-nested multiplex RT-PCR as previously described [11]. Briefly, the entire VP7 gene was first amplified with Beg9/End9 pair of primers and the second amplification was done with 9Con1, 9T-1, 9T-2, 9T-3, 9T-4, and 9T9B set of primers. For VP4 amplification, Con3/Con2 pair of primers and 1T-1, 2T-1, 3T-1, 4T-1, and 5T-1 set of primers were used for the first and second amplification,

respectively. The first-round amplicons of untyped G-types were further analyzed by nested PCR using sets of G12 type-specific primers for the VP7 gene. If positive VP7 and/or VP4 genes first-round amplicons remained untyped, were further sequenced using Beg9/End9 and Con3/Con2 pair of primers, respectively. For global quality assurance, 25% of different strains for VP7 and VP4 genes were randomly selected for partial nucleotide sequencing and further confirmation using the BLAST program [15]. Also, we amplified and sequenced the 11 gene segments from a randomly selected subset of representative samples of the unusual G/P strains to obtain additional information on the genetic backbone as previously described [16]. Genotype assignment was performed using the RotaC v2.0 online software tool [17].

2.3 Phylogenetic analyses of VP7 gene from emergent unusual strains

In order to assess more insightful information about the phylogenetic relationships of the G9 and G8 genotypes detected in this study, analyses of the VP7 nucleotide sequences were conducted. Therefore, we selected five representative sequences from each G-type, other Argentinean G8 and G9 sequences previously detected in Argentina, as well as different worldwide relevant strains available in GenBank. BioEdit v7.0.1 was used to perform the alignments [18]. The model of base substitution was estimated using the ModelFinder module from the IQ-TREE web server according to the Akaike Information Criterion. Maximum Likelihood phylogenetic trees were obtained using the IQ-TREE web server, with TPM3u and HKY as substitution models for G9 and G8 strains, respectively, and gamma-distributed rate variation among sites [19]. Ultrafast Bootstrap approximation (UFBoot; 1000 replicates) was used for phylogenetic grouping branch support [20]. All the VP7 genes sequenced in this study for phylogenetic analyses were submitted to GenBank and assigned the following accession numbers MN921184-921185 (G8 strains detected in 2010 associated with P[6]), MN921193-921197 (G8 strains detected in 2018 associated with P[8]), MN921186-921187 (G9 strains detected in 2016 associated with P[8]), and MN921188-921192 (G9 strains detected in 2017 associated with P[4]). This study was approved by the Ethics Committee of Instituto Nacional de Enfermedades Virales Humanas INEVH-ANLIS “Dr. Julio I. Maiztegui”.

3. Results

3.1 Prevalence and seasonality of laboratory-confirmed rotavirus cases

During 2017–2018, 1183 rotavirus cases (13.2%) were detected among 8976 tests and no significant differences were observed between both years. Most of the tests were run for children under 1 year old, but prevalence was uniform among the different under-5-years-old age subgroups (Table 1). Regarding weekly distribution, the increase of confirmed cases was observed around epidemiological week 35 (late August and early September) for both years. In 2017 this increase lasted until the end of November with uneven peaks. Conversely, only one definite peak was observed around EWs 35–37 in the next year (Fig. 1).

Table 1
Rotavirus frequency of detection and age group distribution in Argentina, 2017–2018

	2017				2018			
Age[a]	No. of tests	RVA (+)	%	RFP	No. of tests	RVA (+)	%	RFP
< 1 year	1996	247	12.4	42.2	1754	227	12.9	41.3
1 year	1337	181	13.5	28.3	1300	166	12.8	30.6
2–4 years	1392	191	13.7	29.5	1197	171	14.3	28.1
Total	4725	619	13.1	100.0	4251	564	13.3	100.0

[a]<1 y indicates under 1 year; RFP, relative frequency proportion.

3.2 Group A rotavirus circulating genotypes

Of the positive rotavirus samples that some hospital laboratories from the surveillance network were able to send to the National Reference Laboratory, 168 and 171 were suitable for G- and P-genotyping for 2017 and 2018, respectively (which represents ~ 95% of submitted samples for each year). Overall analyses for both years also showed that G1P[8] remained practically undetected and G9P[8] circulated at a constant frequency around 10%. In 2017, G2P[4] (27.4%) and G3P[8] (25.0%) were the most frequently detected strains at moderate rates, followed by G12P[8] (18.5%). Noteworthy, the detection of uncommon G9P[4] genotype, only previously detected in one sporadic case in 2013, was observed at 14.9%. The following year, G2P[4] declined abruptly until practically undetected (0.6%) and G3P[8] also showed a significant decrease (9.4%). G12P[8] genotype increased and became the most frequently detected genotype at a high rate (47.4%). Uncommon G9P[4] strain was not detected during 2018. Surprisingly, G8P[8] was the second most frequently detected genotype, a considered uncommon strain which was never detected in Argentina before. Also, the number of samples in which G- and P- type could not be determined due to lack of amplification of first-round product represented ~ 10% in 2018. Circulating genotype distribution is listed in Table 2.

Table 2
Circulating rotavirus genotypes in Argentina. 2017–2018

RVA genotype	2017[a] (%; n = 168)	2018[a] (%; n = 171)
G1P[8]	1.2	0.0
G2P[4]	**27.4**	0.6
G3P[8]	**25.0**	9.4
G4P[8]	0.5	0.0
G8P[8]	0.0	18.1
G9P[4]	14.9	0.0
G9P[8]	8.3	8.8
G12P[8]	18.5	**47.4**
Other G/P types[b]	3.0	5.8
Mixed infections	0.0	0.0
Untypeable strains[c]	1.2	9.9
Total	100.0	100.0

[a] Most prevalent genotypes for each year are in bold and uncommon emergent strains are underlined.

[b] 'Other G/P types' category includes samples that were partially G/P typed (i.e., GxP[NT] and GNTP[x]) and G/P associations that were only sporadically detected (frequency of detection below 0.2%).

[c] 'Untypeable strains' category represents those rotavirus positive samples by ELISA or immunocromatography in which first-round amplicons could not be amplified for further genotyping nor sequencing.

3.3 Genomic and phylogenetic analysis of unusual emergent strains

Additional genetic background analyses (near full genome genotyping) from a randomly selected subset of representative samples showed that the unusual emergent strains bore the G9P[4]-I2-R2-C2-M2-A2-N2-T2-E6-H2 and G8P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2 constellations, respectively.

Phylogenetic analyses of VP7 gene from G9 strains showed that they belonged to major lineage III, and clustered with other G9 strains bearing P[4] genotype. Noteworthy, Argentinean strains were more closely related to the G9P[4] strains detected in Italy during 2016 and India during 2009–2013 than to the ones that circulated in Latin America in 2009–2010 (Fig. 2a). The strains detected in this study showed 99.3% of identity at both nucleotide and amino acid level with Italian strains, and 99.1% and 99.0% with Indian

strains at nucleotide and amino acid level, respectively. Conversely, 98.2% and 98.5% of identity at nucleotide and amino acid level respectively, was observed between Argentinean and Latin American G9P[4] strains. Also, comparison of VP7 gene among emergent strains reported in this study and G9P[8] strains recently circulating in Argentina in 2016 revealed 96.1% and 96.5% of identity at nucleotide and amino acid level, respectively. Analyses of the VP7 antigenic sites revealed that few amino acid changes were detected among the Argentinean strains G9P[4] reported in this study: 2 with Latin American strains (D100N and S221N), 1 with Argentinean G9P[8] from 2016 (S221N) and no changes with the Italian and Indian strains (data not shown).

On the other hand, VP7 gene analyses of G8 strains showed that they belonged to lineage IV and clustered with other G8P[8] strains that have been circulating worldwide since 2013 (Fig. 2b). The most closely related strains were those reported in South Korea in 2017 (99.7% of identity at both nucleotide and amino acid level) and Chile in 2016 (99.5% and 99.2% of identity at nucleotide and amino acid level, respectively). Regarding antigenic sites, no changes in the VP7 deduced amino acid sequences were observed among the Argentinean and the South Korean and Chilean strains (data not shown). In Argentina, G8 strains infecting humans were only detected sporadically in association with P[6] in 2010. However, phylogenetic analyses of VP7 gene showed that these strains belonged to lineage V (Fig. 2b) and 86.2% and 91.7% of identity at nucleotide and amino acid level, respectively.

4. Discussion

Argentina was one of the latest countries to adopt universal rotavirus vaccination in the Americas. However, rapid and successful results were observed regarding burden of diarrheal disease [14]. Following four years of this strategy, the prevalence of rotavirus remained low in children under 5 years of age in comparison with the previous study [14]. These findings oppose to other experiences that had described an increase in the laboratory confirmed cases every two years [21]. Also, the frequency of detection in older age subgroups (i.e., 2–4 years) was slightly higher than in the under 1 year old. This could be explained by the fact that the number of older susceptible children in the ongoing years since the vaccine introduction is still high.

Temporal distribution of laboratory confirmed rotavirus cases showed fluctuations. Thus, it seems difficult to determine a clear seasonal pattern because minor differences in the absolute number of cases may influence the results of this distribution given that the detection frequency of rotavirus has decreased significantly. Nonetheless, the largest amount of weekly cases was observed to be shifted to the spring season in comparison with the pre vaccination period.

Regarding molecular characterization analyses, co-circulation and annual fluctuation were observed. The genotypes G2P[4] and G3P[8], which were the most prevalent in 2016 after vaccine introduction, showed downward trend. Noteworthy, G2P[4] sharply decreased before becoming almost undetected. On the other hand, G12P[8] increased and accounted for around half of the circulating genotypes and G9P[8] has been persistently detected around 8–9% by 2018. It is important to highlight the increase in non-typeable

strains reaching around 10% in 2018. These samples have been confirmed by ELISA but with our available molecular methods they could not further amplify VP7 or partial VP4 genes. Thus, some of these could have been false positive samples. Considering that viral diversity has been reported to increase in those countries with a post-vaccination setting [7], it would be interesting to study these samples with a more sensitive PCR workflow to verify results from antigen testing in the future. Also, current genotyping strategies should be updated more regularly based on the circulating strains [22]. Furthermore, if full genome genotyping cannot be performed for all the positive rotavirus samples, it would be useful to implement the genotyping of other gene segments (i.e., VP6, NSP4) to provide additional information of the genomic constellations that are circulating [23, 24].

The most outstanding findings in this study were the detection of two uncommon rotavirus strains at moderate frequencies that had circulated very sporadically or even had never been identified before. In the Americas, the G9P[4] genotype was sporadically reported in several countries, such as Brazil and the United States [8, 25]. On the other hand, Guatemala, Honduras, Mexico, Colombia and Bolivia have detected these strains at a relative frequency greater than 10% in the last decade [26–28]. However, phylogenetic analyses showed that Argentinean G9P[4] were more closely related to certain strains recently detected in Italy and India [29, 30] than to the ones circulating in our region.

The G8 genotype has been found in cattle and other species of the *Artiodactyla* order [31, 32]. In general, it has been highly prevalent in different countries in Africa but recently has also been detected in South Asia and Europe [33–36]. In Latin America, it has been sporadically detected in humans in association with P[4] and P[6] in Brazil, P[6] in Argentina, P[14] in Venezuela, and P[1] in Paraguay, these latter two related with interspecies transmission [37–39]. As G8P[8], it was identified in several countries of Central America and recently in Chile during a community diarrheal surveillance [40, 41]. Identity analyses of Argentinean G8P[8] detected in this study showed that they were more closely related to South Korean strains detected in 2017 [Truong et al., unpublished] than to those circulating in the neighboring Chile.

All things considered, in both cases our initial findings suggest that the emergence of G9P[4] and G8P[8] strains could be due to the introduction of new strains, rather than to reassortment events from G9P[8] or G8P[6] strains previously circulating in our country. Although more nucleotide sequences data from Latin American countries are required to understand the origin of these strains' introduction, these analyses provide useful evidence for the hypothesis that rotavirus geographical spread dynamic is more complex than we can estimate.

While there has been a sharp decrease of G2P[4], the uncommon strains detected at moderate frequencies bore the genotype 2 genomic constellation. This observation can be explained by the depletion of population of individuals susceptible to G2P[4] after the sudden increase of this genotype in the first years after universal vaccination [14]. However, other strains that are more antigenically distant from the monovalent vaccine strain may still persist and prevail among residual cases. The high frequency of detection of these strains also suggests more efficient spreading mechanisms than other

unusual G/P genotypes, and even than other G9P[4] and G8P[6] previously described. Thus, this may represent a starting point for further studies of adaptability in some uncommon strains.

Considering that the epidemiology of rotavirus has changed, it is important to support ongoing surveillance to assess whether the outcomes of this intervention remain stable, with a specific focus on the genotype diversity to add evidence for evaluation of current immunization strategies and to contribute in the development of new vaccines.

5. Conclusions

In this study we assessed the impact of universal rotavirus vaccination in laboratory-confirmed cases and genotype distribution in Argentina during 2017–2018. Since vaccine introduction, the frequency of detection of rotavirus cases reduced around 50% comparing to the pre vaccination period. Noteworthy, two uncommon strains (G9P[4] and G8P[8]) were detected at relatively high frequency. Phylogenetic analyses revealed that these VP7 nucleotide sequences were more related to strains circulating in Europe and South Asia than to others circulating in the American region. Continuous rotavirus monitoring proves to be necessary for evaluation of current immunization strategies.

Abbreviations

EW
epidemiological week
SNVS 2.0
Argentinean Health Surveillance System

Declarations

Acknowledgments

* Here are listed the members of Argentinean Rotavirus Surveillance Network that contributed in this study:

1. L. Benvenutti (Htal. Penna, Buenos Aires); M. F. Bulgheroni (Htal. Heller, Neuquén); G. Cabral (Htal. Posadas, Buenos Aires); F. Canna (Laboratorio Central, Córdoba); N. Cech (Htal. 4 de Junio, Chaco); P. Cortes (Htal. del Niño Jesús, Córdoba); V. Eibar (Htal. Notti, Mendoza); L. Fierro (Htal. Rawson, San Juan); M. Figueredo (Htal Perón, Formosa); S. Flores (Htal. Eva Perón, Tucumán); L. López (Htal. Durand, CABA); E. Lozano (Htal Quintana, Jujuy); N. Lucero (Htal. Schestakow, Mendoza); J. Palau (Htal. Sor María Ludovica, Buenos Aires); M. Roncallo (Htal. Cipolletti, Río Negro); I. Silveyra (Htal. Centeno, La Pampa); G. Sucin (Htal. Castelán, Chaco); A. Zurschmitten (Htal. Junín de los Andes, Neuquén).

Author contribution

JID and JAS participated in the conceptualization of the work. ARSN provided stool samples. JID developed the methods, analyzed and interpreted the data. JAS analyzed and interpreted the data. JID and JAS contributed to writing the manuscript. All authors read and approved the final manuscript.

Funding

None to declare.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Instituto Nacional de Enfermedades Virales Humanas INEVH-ANLIS “Dr. Julio I. Maiztegui”.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the GenBank database.

Competing interests

The authors declare that they have no competing interests.

References

1. Aliabadi N, Antoni S, Mwenda JM, Weldegebriel G, Biey JNM, Cheikh D, et al. Global impact of rotavirus vaccine introduction on rotavirus hospitalisations among children under 5 years of age, 2008-16: findings from the Global Rotavirus Surveillance Network. Lancet Glob Health. 2019;7:e893–903.
2. Chavers T, De Oliveira LH, Parashar UD, Tate JE. Post-licensure experience with rotavirus vaccination in Latin America and the Caribbean: a systematic review and meta-analysis. Expert Rev Vaccines. 2018;17:1037–51.
3. Matthijnssens J, Nakagomi O, Kirkwood CD, Ciarlet M, Desselberger U, Van Ranst M. Group A rotavirus universal mass vaccination: how and to what extent will selective pressure influence prevalence of rotavirus genotypes? Expert Rev Vaccines. 2012;11:1347–54.
4. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol. 2005;15:29–56.
5. Banyai K, Laszlo B, Duque J, Steele AD, Nelson EAS, Gentsch JR, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for

understanding the impact of rotavirus vaccination programs. Vaccine. 2012;30(Suppl 1):A122–30.

6. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol. 2008;82:3204–19.
7. Roczo-Farkas S, Kirkwood CD, Cowley D, Barnes GL, Bishop RF, Bogdanovic-Sakran N, et al. The Impact of Rotavirus Vaccines on Genotype Diversity: A Comprehensive Analysis of 2 Decades of Australian Surveillance Data. J Infect Dis. 2018;218:546–54.
8. Bowen MD, Mijatovic-Rustempasic S, Esona MD, Teel EN, Gautam R, Sturgeon M, et al. Rotavirus Strain Trends During the Postlicensure Vaccine Era: United States, 2008–2013. J Infect Dis. 2016;214:732–8.
9. Iturriza-Gómara M, Dallman T, Bányai K, Böttiger B, Buesa J, Diedrich S, et al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. Epidemiol Infect. 2011;139:895–909.
10. Degiuseppe JI, Giovacchini C, Stupka JA. Red Nacional de Vigilancia de Gastroenteritis Virales. [Rotavirus epidemiology and surveillance in Argentina: 2009–2011]. Arch Argent Pediatr. 2013;111:148–54.
11. Degiuseppe JI, Reale EA, Stupka JA, Argentine Rotavirus Surveillance Network. Rotavirus epidemiology and surveillance before vaccine introduction in Argentina, 2012–2014. J Med Virol. 2017;89:423–8.
12. Stupka JA, Carvalho P, Amarilla AA, Massana M, Parra GI, Argentinean National Surveillance Network for Diarrheas. National Rotavirus Surveillance in Argentina: high incidence of G9P[8] strains and detection of G4P[6] strains with porcine characteristics. Infect Genet Evol. 2009;9:1225–31.
13. Stupka JA, Degiuseppe JI, Parra GI, Argentinean National Rotavirus Surveillance Network. Increased frequency of rotavirus G3P[8] and G12P[8] in Argentina during 2008–2009: whole-genome characterization of emerging G12P[8] strains. J Clin Virol. 2012;54:162–7.
14. Degiuseppe JI, Stupka JA. First assessment of all-cause acute diarrhoea and rotavirus-confirmed cases following massive vaccination in Argentina. Epidemiol Infect. 2018;146:1948–54.
15. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
16. Matthijnssens J, Ciarlet M, Rahman M, Attoui H, Bányai K, Estes MK, et al. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol. 2008;153:1621–9.
17. Maes P, Matthijnssens J, Rahman M, Van Ranst M. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 2009;9:238.
18. Hall T. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999;41:95–8.
19. Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016;44:W232–5.

20. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol Biol Evol. 2018;35:518–22.
21. Hallowell BD, Parashar UD, Curns A, DeGroote NP, Tate JE. Trends in the Laboratory Detection of Rotavirus Before and After Implementation of Routine Rotavirus Vaccination - United States, 2000–2018. MMWR Morb Mortal Wkly Rep. 2019;68:539–43.
22. Esona MD, Gautam R, Tam KI, Williams A, Mijatovic-Rustempasic S, Bowen MD. Multiplexed one-step RT-PCR VP7 and VP4 genotyping assays for rotaviruses using updated primers. J Virol Methods. 2015;223:96–104.
23. McKell AO, Nichols JC, McDonald SM. PCR-based approach to distinguish group A human rotavirus genotype 1 vs. genotype 2 genes. J Virol Methods. 2013;194:197–205.
24. Thongprachum A, Chaimongkol N, Khamrin P, Pantip C, Mizuguchi M, Ushijima H, et al. A novel multiplex RT-PCR for identification of VP6 subgroups of human and porcine rotaviruses. J Virol Methods. 2010;168:191–6.
25. Luchs A, Cilli A, Morillo SG, Carmona R, de CC, Timenetsky M do CST. Rotavirus genotypes circulating in Brazil, 2007–2012: implications for the vaccine program. Rev Inst Med Trop Sao Paulo. 2015;57:305–13.
26. Quaye O, McDonald S, Esona MD, Lyde FC, Mijatovic-Rustempasic S, Roy S, et al. Rotavirus G9P[4] in 3 countries in Latin America, 2009–2010. Emerging Infect Dis. 2013;19:1332–3.
27. McAtee CL, Webman R, Gilman RH, Mejia C, Bern C, Apaza S, et al. Burden of Norovirus and Rotavirus in Children After Rotavirus Vaccine Introduction, Cochabamba, Bolivia. Am J Trop Med Hyg. 2016;94:212–7.
28. Peláez-Carvajal D, Cotes-Cantillo K, Paternina-Caicedo A, Gentsch J, de la Hoz-Restrepo F, Patel M. Characterization of rotavirus genotypes before and after the introduction of a monovalent rotavirus vaccine in Colombia. J Med Virol. 2014;86:1083–6.
29. Ianiro G, Recanatini C, D'Errico MM, Monini M, RotaNet-Italy Study Group. Uncommon G9P[4] group A rotavirus strains causing dehydrating diarrhea in young children in Italy. Infect Genet Evol. 2018;64:57–64.
30. Doan YH, Suzuki Y, Fujii Y, Haga K, Fujimoto A, Takai-Todaka R, et al. Complex reassortment events of unusual G9P[4] rotavirus strains in India between 2011 and 2013. Infect Genet Evol. 2017;54:417–28.
31. Louge Uriarte EL, Badaracco A, Matthijnssens J, Zeller M, Heylen E, Manazza J, et al. The first caprine rotavirus detected in Argentina displays genomic features resembling virus strains infecting members of the Bovidae and Camelidae. Vet Microbiol. 2014;171:189–97.
32. Parreño V, Bok K, Fernandez F, Gomez J. Molecular characterization of the first isolation of rotavirus in guanacos (Lama guanicoe). Arch Virol. 2004;149:2465–71.
33. Yodmeeklin A, Khamrin P, Kumthip K, Malasao R, Ukarapol N, Ushijima H, et al. Increasing predominance of G8P[8] species A rotaviruses in children admitted to hospital with acute gastroenteritis in Thailand, 2010–2013. Arch Virol. 2018;163:2165–78.

34. Moutelíková R, Sauer P, Dvořáková Heroldová M, Holá V, Prodělalová J. Emergence of Rare Bovine-Human Reassortant DS-1-Like Rotavirus A Strains with G8P[8] Genotype in Human Patients in the Czech Republic. Viruses. 2019;11.
35. Kondo K, Tsugawa T, Ono M, Ohara T, Fujibayashi S, Tahara Y, et al. Clinical and Molecular Characteristics of Human Rotavirus G8P[8] Outbreak Strain, Japan, 2014. Emerging Infect Dis. 2017;23:968–72.
36. Hoa-Tran TN, Nakagomi T, Vu HM, Do LP, Gauchan P, Agbemabiese CA, et al. Abrupt emergence and predominance in Vietnam of rotavirus A strains possessing a bovine-like G8 on a DS-1-like background. Arch Virol. 2016;161:479–82.
37. Montenegro FMU, Correia JB, Rodrigues Falbo A, Dove W, Nakagomi T, Nakagomi O, et al. Anticipating rotavirus vaccines in Brazil: detection and molecular characterization of emerging rotavirus serotypes G8 and G9 among children with diarrhoea in Recife, Brazil. J Med Virol. 2007;79:335–40.
38. Martinez M, Phan TG, Galeano ME, Russomando G, Parreno V, Delwart E, et al. Genomic characterization of a rotavirus G8P[1] detected in a child with diarrhea reveal direct animal-to-human transmission. Infect Genet Evol. 2014;27:402–7.
39. Vizzi E, Piñeros O, González GG, Zambrano JL, Ludert JE, Liprandi F. Genotyping of human rotaviruses circulating among children with diarrhea in Valencia, Venezuela. J Med Virol. 2011;83:2225–32.
40. Lucero Y, O'Ryan M, Liparoti G, Huerta N, Mamani N, Ramani S, et al. Predominance of Rotavirus G8P[8] in a City in Chile, a Country Without Rotavirus Vaccination. J Pediatr. 2019;204:298–300.e1.
41. de Oliveira LH, Danovaro-Holliday MC, Sanwogou NJ, Ruiz-Matus C, Tambini G, Andrus JK. Progress in the introduction of the rotavirus vaccine in Latin America and the Caribbean: four years of accumulated experience. Pediatr Infect Dis J. 2011;30:61–6.

Figures

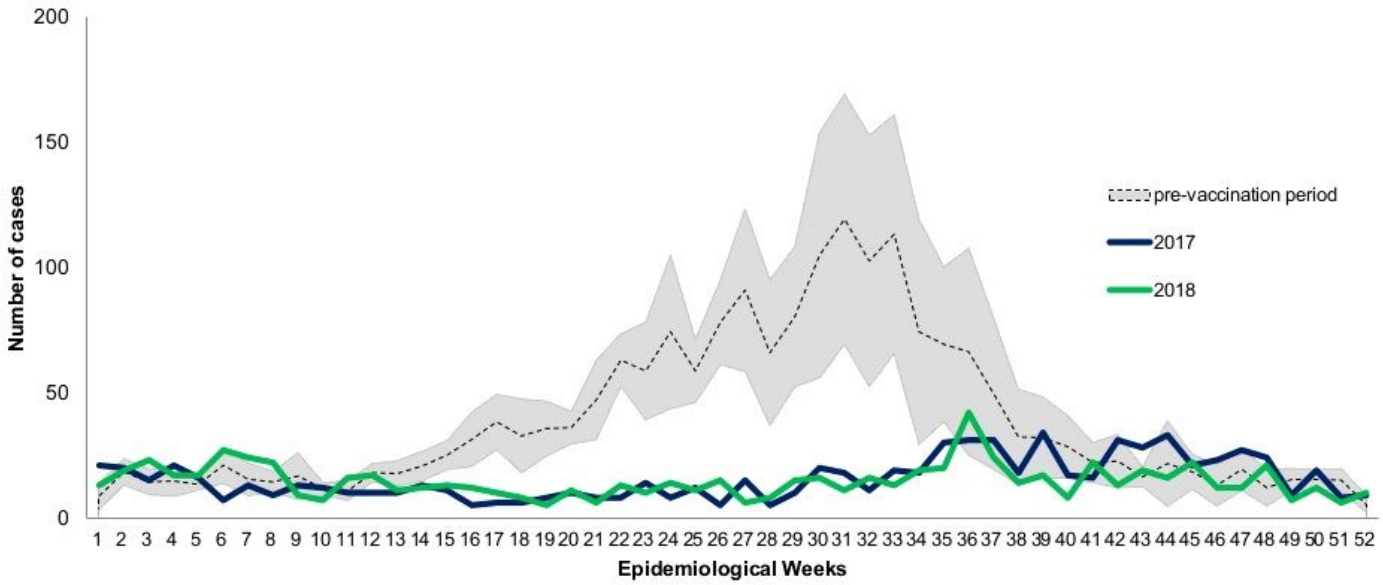

Figure 1

Weekly distribution of laboratory-confirmed rotavirus cases in children under 5 years of age in Argentina, pre-vaccination period (2012-2014, mean and range), 2017, and 2018. Lines indicate the number of rotavirus-confirmed cases notified to SNVS 2.0 according to epidemiological week (EW) (reference code provided).

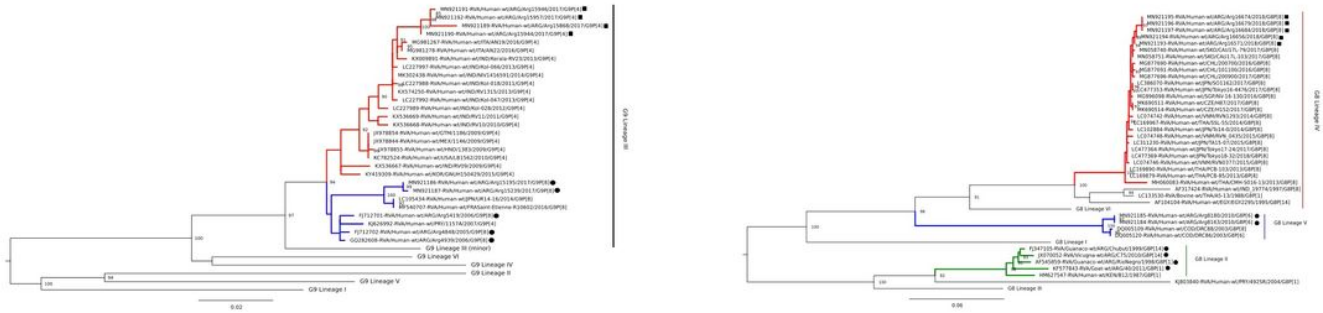

Figure 2

Phylogenetic analysis of Argentinean G9P[4] and G8P[8] strains detected during 2017-2018. Maximum-likelihood phylogenetic trees of rotavirus VP7 gene of Argentinean (A) G9P[4], (B) G8P[8] and other relevant strains were created by using the TPM3u and HKY models, respectively and gamma-distributed rate variation among sites. Ultrafast Bootstrap values (>85) from 1,000 replicates are shown on the nodes. GenBank accession numbers are shown. Scale bars indicate genetic distance (nucleotide substitutions/site). (A) G9P[4] strains detected in this study are marked with a filled square and previous G9P[8] previously circulating in Argentina with a filled circle. (B) Argentinean G8P[8] strains detected in this study are marked with a filled square and other G8 previously circulating in Argentina with a filled

circle. In both cases, clusters in which Argentinean G9 and G8 strains grouped are indicated in different colors.