



Epidemiological, molecular, and clinical features of rotavirus infections among pediatrics in Qatar

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Abstract

Acute gastroenteritis (AGE) remains a major cause of diarrhea in developing and developed countries. Rotavirus (RV) is a leading cause of severe pediatric diarrhea worldwide. Here we report on the prevalence of circulating genotypes in association with demographics and clinical manifestations outcomes in Qatar. A total of 231 RV-positive fecal samples were collected from children suffering from AGE during 3 years study period between June 2016 and June 2019. The age of the subjects ranged between 2 months and 14 years (median of 16 months). The VP4 and VP7 were amplified and sequenced. Phylogenetic analyses were performed using MEGA7.0. Pearson's chi-squared test was used to determine significant differences for comparisons of general categorical variables. RV infections were most common in children between 1 and 3 years of age (49%), followed by those < 1 year and > 3 years of age (33% and 28%, respectively). RV infections were more frequent in males than females, with a ratio of 1.4:1. RV infections occurred throughout the year, with a noticeable increase in summer (42.8%) and a drop in winter (20.1%). RV genotypes G3P[8] (30.8%), G2P[8] (12.3%), G4P[8] (11.7%), and G1P[8] (10.4%) were the common genotypes during the study period. The G3P[8] strain detected in our study revealed similarities to the equine-like G3P[8] (10.3%; 24/231) (KT988229.1), Wa-like genomic constellation (9%; 21/231) (MF563894.1), and DS-1-like strains (6.4%; 15/231) (LC386081.1). Based on the Vesikari score system, severe clinical illness including diarrhea and vomiting (average frequency: 4 to 5 times/day) was recorded for G3P[8] group, followed by G9P[8], G4P[8], and G1P[8]. Higher incidence for G3P[8], G2P[8], G4P[8], and G1P[8] were reported in Qatari subjects compared to other nationalities. The multinational status of a small country explains the wide diversity of circulating RV genotypes in Qatar. The highest prevalence and severe illnesses were recorded to G3P[8], which is different from other surrounding countries/global levels.

Keywords Rotavirus · Genotyping · Vaccination · Age-specific

Introduction

Rotavirus (RV) infections are a leading global cause of severe gastroenteritis (GE) and severe diarrheal disease among

infants and children worldwide [1]. According to the 2016 World Health Organization (WHO) estimates, around 215,000 children aged under 5 years die from vaccine-preventable rotavirus infections every year [2]. Although the incidence rate of RV infections represents a high burden in both low- and high-income countries, the mortality rate due to RV infections is higher in low-income countries [3]. It is estimated that by the age of 5 years, most children will have had at least one episode of RV infections [4]. The primary source of the transmission of these viruses is through the fecal-oral route and possibly by contaminated surfaces, and hands [5]. Clinical symptoms associated with RV infections are diarrhea, vomiting, and fever in infants and young children.

RV, a member *Reoviridae* family, is a non-enveloped wheel-like double-stranded RNA virus with 11 viral genome segments [6] encoding for six non-structural proteins (NSP1-NSP6) that are involved in viral virulence and six structural viral proteins

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(VP1–VP4, VP6, and VP7), which make up the virus particles [7]. A dual classification system has been used for typing RV based on the genes coding for the two viral surface proteins: VP7 (G type, glycosylated) and VP4 proteins (P type, protease-sensitive) [8]. Both viral surface proteins are the target for neutralizing antibodies [9]. Because the two genes that determine G types and P types can be passed on separately via reassortment to progeny viruses, different combinations have been reported. Currently, there are 36 different G and 51 different P genotypes with an intratypic variation (<https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg>).

The four common G types (G1–G4), along with G9 and G12, in association with P[6] or P[8], comprise the common circulating rotavirus genotypes globally [10]. The most important strains causing the majority of infections worldwide are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [11]. Currently, available data indicates fluctuations in rotavirus G/P type combinations by geography and season. Further, the emergence of unusual types, such as G5, G6, G8, G9, G12, and P[6], have been reported, but reasons for their emergence remain poorly understood [11].

In order to reduce the high disease burden associated with RV diarrhea, the WHO recommends the use of one of the four oral, live, attenuated (Rotarix™; RotaTeq™; Rotavac™; and RotaSii™) vaccines [12]. Currently, the two oral vaccines *Rotarix*™ (used in Qatar) and *RotaTeq*® are licensed in different parts of the world based on the good safety and efficacy profiles in large global studies [12–18]. These vaccines have shown high efficacy against the globally commonly circulating G types (G1, G2, G3, G4, G9) and P types (P[4], P[6], P[8]) that are known to cause approximately 90% of the disease [10]. Regardless of the lower efficacy in low-income countries, the introduction of these vaccines into national immunization programs has been associated with a significant decline in age-related infantile morbidity and mortality in Europe, Latin America, and Africa [19].

Qatar is known to be a multinational country, where more than 80% of its population are expats arriving from more than 60 countries around the globe [20]. Data about RV in Qatar is limited to one study conducted in 2013, which reported around 10.4% prevalence of the virus among children and adults with AGE illness [21]. However, genetic characterization and the association between circulating genotypes and clinical outcomes have not been studied [21]. In this regard, we report the incidence of the RV infection and the prevalence of G and P serotypes among children in Qatar for 3 years.

Materials and methods

Sample collection

RV-positive stool samples were collected from children admitted to the Pediatric Emergency Center (PEC) of Hamad

Medical Corporation (HMC; the major hospital in Qatar), with signs of AGE (frequent diarrhea and vomiting (> 2) in last 24 h) between 2016 and 2019. IRB approval for the study was obtained from Hamad Medical Corporation (#16173/16) and written informed consent was obtained from the parents of the children to use their samples in the study. In addition to the samples, we also collected demographics (age, gender, and nationality) and clinical data of the enrolled children. The clinical data sheet included information about fever, duration/frequency of both diarrhea and vomiting, date of symptoms onset, admission and discharge dates, antibiotics and other treatments, rotavirus vaccination, neurological symptoms, degree of dehydration, and underlying illnesses. All RV-positive children ($n = 231$) were followed up to 7 days, and their clinical manifestations were reported.

Sample processing and viral nucleic acid extraction

The stool specimens were initially screened with a Film Array Gastrointestinal (GI) Panel kit (BIOFIRE®, Cambridge, USA) at HMC, and samples positive for RV were then transferred to Qatar University (QU) Biomedical Research Center (BRC) for further molecular characterization. Half a gram of a stool sample was suspended in 4 mL of sterile 10% phosphate-buffered saline (PBS) in water. The fecal suspension was then vortexed and centrifuged for 20 min at 4000×g. The supernatant was collected and transferred into a new 15-mL tube before centrifuged again for 1500×g for 10 min, following which, 240 µL of the supernatant was used for viral RNA extraction using a QIAamp Viral RNA extraction kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Concentrations of extracted viral RNA were measured using the Infinite 200 PRO NanoQuant Plate™ - Tecan (NanoQuant, Woburn MA, USA). Reverse transcriptase (RT)–polymerized chain reaction (PCR) amplification of complete VP7 gene (~810 bp) was generated using primers VP7-1 (FP-ATGTATGGTATTGAATATACCAC) and VP7-2 (RP-AACTTGCCACCATTTTTTCC) for VP7 (G type) [22]; and partial VP4 fragment (630 bp) using VP4-1 (FP-TATGCTCCAGTNAATTGG) and VP4-2 (RP-ATTGCATTTCTTTCCATAATG) for VP4 (P type) region [22]. Briefly, 4 µL of purified viral RNA was used as a template in a 20-µL total reaction volume using a Qiagen One-step RT-PCR Kit (QIAGEN, Hilden, Germany). A pre-RT step was carried out at 50 °C for 30 min for reverse transcription, followed by initial PCR activation step at 95 °C for 10 min; denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s (35 cycles); and final extension at 72 °C for 10 min. The amplified products were detected by gel electrophoresis on a 1.5% agarose gel, containing 4 µL of ethidium bromide, using the BioRad. PCR products were stored at –20 °C until further analysis.

RV genotyping

Purification of the PCR product was performed using manual PCR, clean up steps as previously described (http://www.fhrc.org/science/labs/hahn/methods/mol_bio_meth/Big%20Dye%20Protocol.pdf). Briefly, PCR products were added to 27.5 μL ethanol, 1.5 μL of sodium acetate (3 M, pH: 5.2), and 1.5 μL of EDTA (0.5 M, pH: 8), mixed by vortexing and centrifuged at maximum speed for 30 min. The supernatant was discarded, and the PCR purification was done by adding 100 μL of 70% ethanol to each sample, which was vortexed and centrifuged at maximum speed for 10 min. The supernatant was discarded, and samples were air-dried at room temperature overnight before resuspending with 15 μL of nuclease-free water. Nucleotide sequencing of the purified product was performed at Macrogen (Seoul, Korea) using the PCR primers. The obtained sequences were utilized to determine the genotype using the web-based RotaC2.0 automated genotyping tool for rotaviruses [23]. Multiple sequence alignments were done using CLUSTALW, and phylogeny trees were constructed with Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA 7) software using the neighbor-joining approach validated by replicating with 1000 bootstraps as previously reported [24]. The reference strains to demonstrate the relationships between RV genotypes were obtained from the GenBank database.

Vesikari score system for severity

We evaluated the disease severity of RV-infected children by applying the Vesikari score system [25] according to clinical manifestations: total score < 7 considered mild; $7 \leq$ scores ≤ 10 considered moderate; and score > 10 (up to 20) considered severe. The scoring system considered general AGE symptoms: duration of diarrhea and vomiting episodes, temperature, and dehydration (mild dehydration: treat at home; moderate dehydration: treat using oral rehydration salts, 1–5% loss bodyweight, and severe dehydration: treat using IV therapy, $\geq 6\%$ loss body weight) [25]. During the follow-up period (7 days), the following medications were prescribed: electrolyte replacement solution, paracetamol, cefixime, domperidone, NaCl, IVF, salbutamol, and ibuprofen.

Statistical analysis

Pearson's chi-squared test was used to determine significant differences for comparisons of general categorical variables. All statistical data analysis was done by using GraphPad (Prism version 5.04) (IBM, Armonk, NY, USA).

Results

Demography and clinical manifestation of the study subjects

A total of 812 AGE cases were reported to PEC between May 2016 and June 2019, of which 231 (28.4%) were RV infections. RV detection rate varied and plunged from 47% (109/231) in the year 2017 to 7% (18/231) in the year 2019 (Fig. 1). In an assessment of the RV incidence, RV infection rates were highest in Qataris (36.3%), followed by Pakistani (11.2%) and Egyptians (11.2%), respectively.

Higher infections were reported in males (58%) compared to females (42%). In terms of age, highest rates of RV infections were reported in children between 1 and 3 years of age (49%), followed by < 1 year (33%), and > 3 years (28%) of age ($P < 0.05$) (Table 1). There was no significant difference in diarrhea and vomiting episodes between RV-infected and non-RV-infected groups (Table 2). On the other hand, fever was reported more in RV-negative patients (74%, 430/581) compared to those with RV infection (54%, 124/231). The majority of RV-infected children had severe symptoms of diarrhea (100%), dehydration (96.5%), vomiting (97%), and fever (54%) (Table 2). Concerning dehydration, only 4.3% of RV-infected children had severe dehydration, whereas about 52.2% and 40% experienced moderate to mild dehydration, respectively (Table 2). The duration of hospitalization of RV-infected children ranged from 2 to 7 days (average: 4 days, SD: 1.23). The highest number of RV hospitalizations occurred between 14 and 22 months old children (52%; 120/231) while the lowest numbers were reported in children between younger than 12 months (34%; 78/231) and > 23 months of age (14%; 33/231). According to the Vesikari

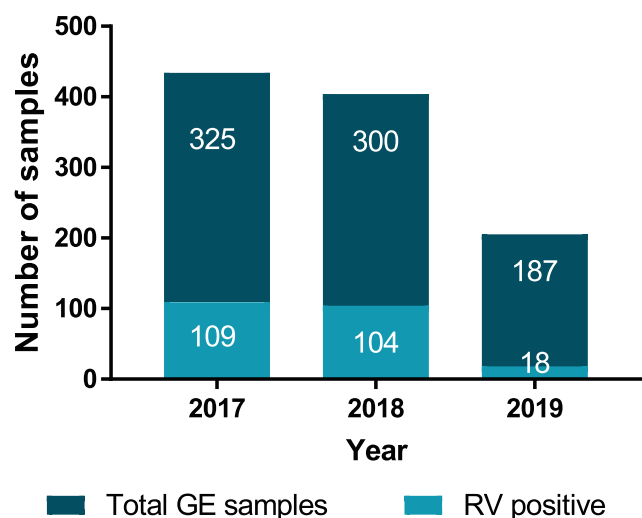


Fig. 1 Overall RV incidence in hospitalized children with AGE. (A) RV incidence rate between May 2016 and June 2019. The number of samples is represented in X axis and the year in Y axis

Table 1 Demographics of study subjects

Variables	May 2016–May 2017 (%)	June 2017–May 2018 (%)	June 2018–May 2019 (%)	Total number of AGE patients (%)	RV-positive (%)	RV-negative (%)	P value
Total number	(n = 325)	(n = 300)	(n = 187)	(n = 812)	(n = 231)	(n = 581)	-
Gender							
Male	193 (59.0)	179 (60.0)	98 (52.0)	486 (60.0)	134 (58.0)	352 (61.0)	<i>P</i> > 0.05*
Female	132 (41.0)	121 (40.0)	89 (48.0)	326 (40.0)	97 (42.0)	229 (39.0)	
Age (years)							
< 1 year	99 (30.0)	98 (33.0)	86 (46.0)	283 (35.0)	77 (33.0)	281 (48.0)	<i>P</i> < 0.05*
1–3 years	161 (50.0)	148 (49.0)	95 (50.0)	404 (50.0)	114 (49.0)	228 (39.0)	
> 3 years	65 (20.0)	54 (18.0)	6 (4.0)	125 (15.0)	40 (28.0)	72 (13.0)	

*Pearson's chi-squared test

score, 55 (23.8%) children reported mild symptoms (score < 7), 68 (29.4%) moderate (7–10), and 108 (46.8%) severe (score > 10). During the follow-up period (7 days), only 17 children were prescribed an electrolyte replacement solution, paracetamol, cefixime, domperidone, NaCl, IVF, salbutamol, and ibuprofen. All the 17 (100%) children were fully recovered by the end of the follow-up period.

Seasonality of RV infections

Seasonal trends of RV infections with temperature and humidity are shown in Fig. 2. AGE cases were reported throughout the year with a marginal increase in the late spring–early summer months, which is associated with temperature increase and drop in humidity. Similarly, a rise in RV cases was reported with the onset of warmer months between April and July. Cumulatively, reported RV cases during these 4 months represented 48.5% of the overall cases. This seasonality pattern was more prominent in the two full years of the study between 2016 and 2018. On the other hand, a drop in RV infections was recorded along with the onset of colder months in Qatar between September and December, comprising 18.2% of the overall infections. A significant drop in AGE cases in general and RV infections in specific was reported between July 2018 and June 2019 (Fig. 2).

Genotypes frequency and diversity

A total of 231 (28.4%) rotavirus-positive were genotyped for G and P by partial sequencing of the VP7 and VP4 genes, respectively. We detected six different rotavirus G genotypes and two different P genotypes, forming eleven different G-P combinations in total. For the G type, G3, G4, and G2 were the predominant types with an incidence of 38%, 20%, and 17%, respectively. On the other hand, P[8], followed by P[4], was the dominant circulating type at an incidence of 71% and 29% during the study period. The predominantly detected RV genotype combination was G3P[8] RV accounting for 31% (*n* = 71) of all strains, followed by G2P[8] for 12.3% (*n* = 28), G4P[8] for 11.6% (*n* = 27), G1P[8] for 10.3% (*n* = 24), G4P[4] for 9% (*n* = 21), G9P[8] for 8.7% (*n* = 20), G3P[4] for 7.3% (*n* = 17), G2P[4] for 5.2% (*n* = 12) and G1P[4] for 2.5% (*n* = 6), G9P[4] for 1.7% (*n* = 4), and G8P[8] for 0.4% (*n* = 1) (Fig. 3).

Based on the phylogeny analysis, two clusters of G3 strains were grouped. The most dominant G3 serotype revealed high similarities (> 98% nucleotide identity) to equine-like G3P[8] RVA strains (GenBank: KT988229.1) that circulated in Italy between 2012 and 2013, Wa-like genomic constellation detected in Eastern India between 2014 and 2016 (GenBank: MF563894.1), and DS-1-like reassortment strains detected in Japan (LC386081.1) (2017–2018) (Fig. 4a). The second most circulating serotype G4 was closely related (> 98% nucleotide

Table 2 Clinical manifestations of study subjects

Variables	Total number of patients (%) (<i>n</i> = 812)	RV-positive (%) (<i>n</i> = 231)	RV-negative (%) (<i>n</i> = 581)
Fever			
Yes	554 (68.0)	124 (54.0)	430 (74.0)
No	258 (32.0)	107 (46.0)	151 (26.0)
Max reached fever (°C)	40.2	40	39.3
Vesikari score	7 ≤ scores ≤ 10	score > 10	7 ≤ scores ≤ 10
Vomiting			
Yes	724 (89.0)	225 (97.0)	499 (86.0)
Frequency (<i>n</i>)	1–5	2–5	1–4
Duration (days)	1–7	2–7	1–7
No	88 (11.0)	6 (3.0)	82 (14.0)
Vesikari score	7 ≤ scores ≤ 10	score > 10	7 ≤ scores ≤ 10
Diarrhea			
Yes	807 (99.0)	231 (100.0)	576 (99.0)
Frequency (<i>n</i>)	1–10	2–10	2–10
Duration (days)	1–10	2–6	1–10
No diarrhea	5 (1.0)	0 (0.0)	5 (1.0)
Vesikari score	7 ≤ scores ≤ 10	score > 10	7 ≤ scores ≤ 10
Dehydration			
Severe	30 (3.7)	10 (4.3)	20 (3.4)
Moderate	325 (40.0)	122 (52.2)	203 (35.0)
Mild	449 (55.3)	91 (40.0)	358 (61.6)
No dehydration	8 (1.00)	8 (3.5)	0 (0.0)
Vesikari score	7 ≤ scores ≤ 10	score > 10	7 ≤ scores ≤ 10
Mean duration of hospitalization (days)	1–7	2–7	1–5
Treatment			
Number	NA	17	NA
Fully recovered	NA	17	NA
Partially recovered	NA	0	NA
Worsening	NA	0	NA
Recovered but became sick again	NA	0	NA
No treatment			
Number	231 (28.5)	208 (92.6)	17 (3.0)
Fully recovered	NA	208 (92.6)	NA
Partial recovered	NA	0	NA
Worsening	NA	0	NA
Recovered but became sick again	NA	0	NA

NA, not available

identity) to the viral strains from Russia/2008 (FJ440333.2) (Fig. 4b). G2 strains were closely related (>99% nucleotide identity) to strains from Pakistan (GenBank: MF673444.1) which circulated during 2015–2016, Turkey (GenBank: MF494809.1) during 2014–2016, and Lebanon (GenBank: MH591274.1) between 2011 and 2018 (Fig. 4c). Meanwhile, predominant P[8] shared more than 98% nucleotide similarity

to Russia/2013 (KX758597.1), Russia/2014 (KY774441.1), and Russia/2016 (KX228858.1) (Fig. 4d).

Seasonality of RV genotypes

Rotavirus serotype G3 was the most prevalent throughout the year except in the month of July (2016), September (2016),

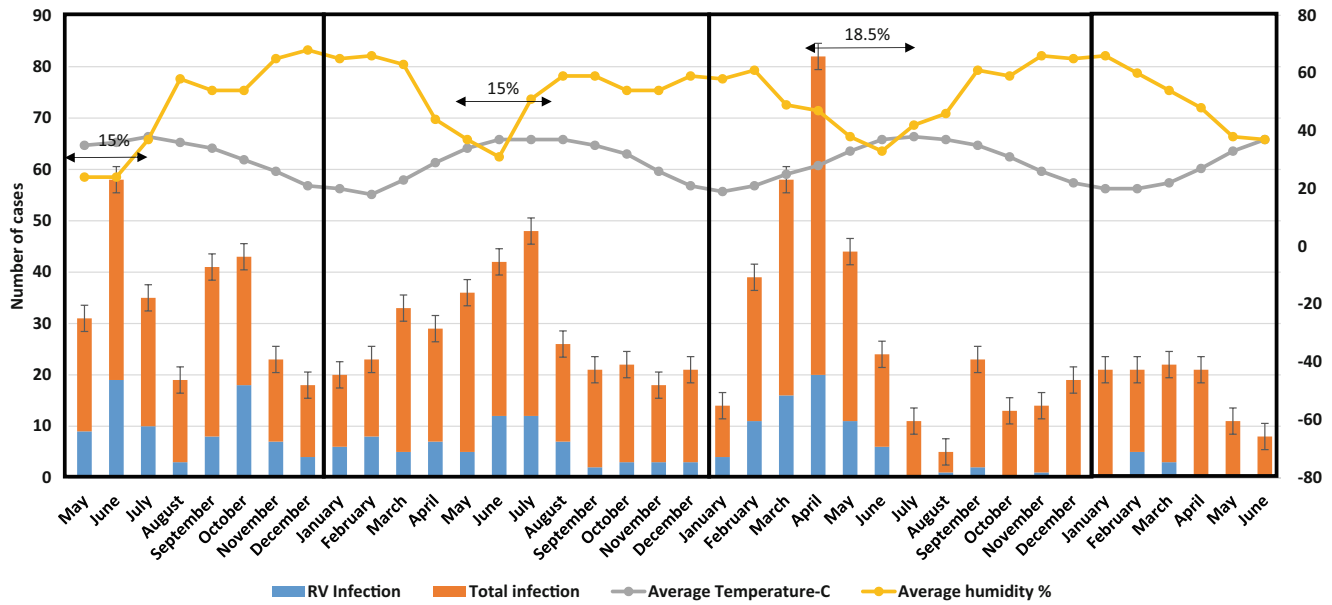


Fig. 2 Stacked bar chart denoting cumulative number of RV-positive cases over total AGE samples tested in Qatar during the study period (May 2016 to June 2019). X axis: month; Y axis: cumulative number of children. Average temperature and average humidity between May 2016

and June 2019 in Qatar are represented as lines. The value on the arrow in the graph states the percentage of RV-positive cases among AGE case in all study periods

December–January (2017–2018), and between September 2018 and May 2019. In the absence of serotype G3, infections with G1, G2, and G9 were more prevalent. Serotypes G1, G2, G4, and G9 were detected throughout the study period at

varying frequencies. The uncommon G8 (G type) was detected in a single sample from a 15-month-old male child (Qatari nationality) patient in 2018. On the other hand, we noticed a significant increase of P[8] following P[4] peaks, which was not season or month specific. In detail, P[4] was detected at varying frequencies during the study period from May 2016 to April 2017 (reaching maximum peak), before the circulation of P[8] in June 2017. Similarly, P[4] gradually increased in January 2018 before the appearance of P[8] in February 2018. Furthermore, both P[4] and P[8] did not show any unique pattern of distribution but differed significantly across seasons (Fig. 5a).

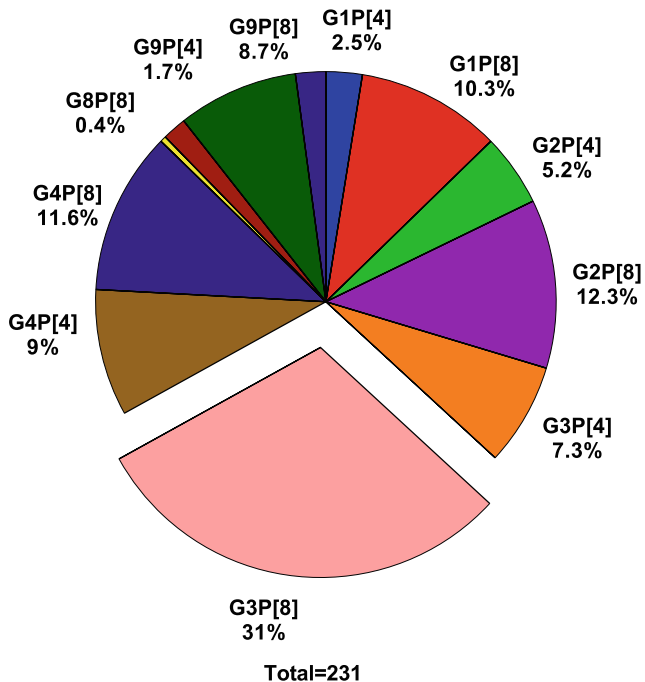


Fig. 3 Frequency of circulating genotypes detected in Qatar between May 2016 and June 2019

Overall, RV genotype predominance differed throughout the study period. Furthermore, G3P[8] was predominant during the winter season (September–November) in 2016; however, its circulation shifted towards the summer season (June–August) in Qatar between 2017 and 2018. As for G2P[4], it was more frequent from June 2016 to October 2017 but did not appear in the following years (Fig. 5b). Besides, G2P[8], G3P[4], G4P[4], and G4P[8] were encountered across the study period with no specific pattern of circulation. Moreover, less predominant G9P[4] was noticed once in the summer season (May 2016) and then later in the winter season (February 2018). The uncommon genotype G8P[8] circulated only in April (warm month) in the year 2018. It is worth noting that we did not have any RV infections during the winter season in 2018 (September–December). Notably, only G1P[4] was detected only in the colder months (January and February) in 2019 (Fig. 5b).

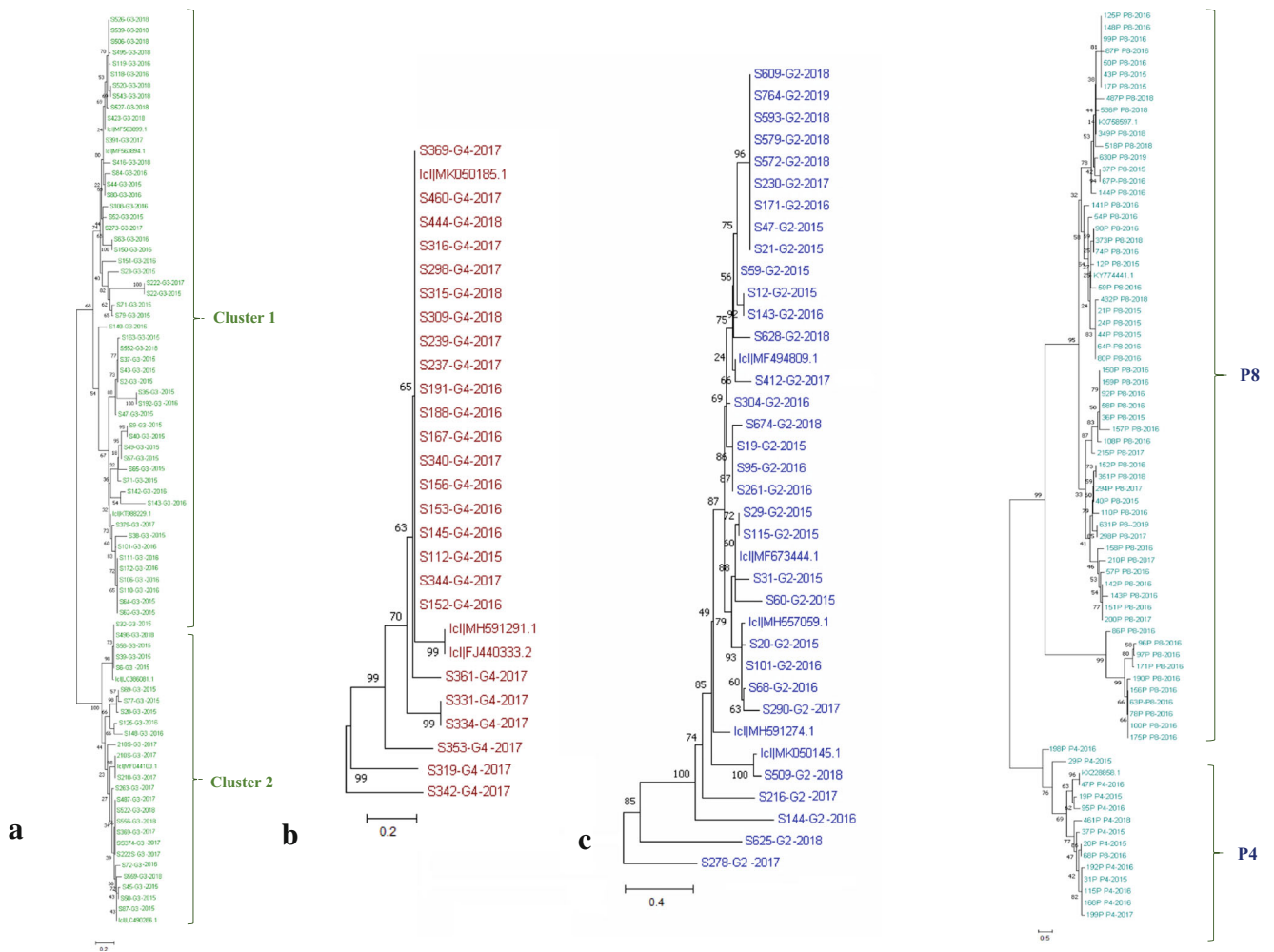


Fig. 4 Phylogeny analysis of RV circulating strains in children with AGE in Qatar. The evolutionary tree was constructed for VP4 region using neighbor-joining method with 1000 bootstrap with MEGA7 [26]. The evolutionary distances were computed using the Poisson correction

method and are in the units of the number of amino acid substitutions per site. **a** RV G3 VP7 coding gene. **b** RV G4 VP7 coding gene. **c** RV G2 VP7 coding gene. **d** RV P[4]/P[8] VP4 coding gene

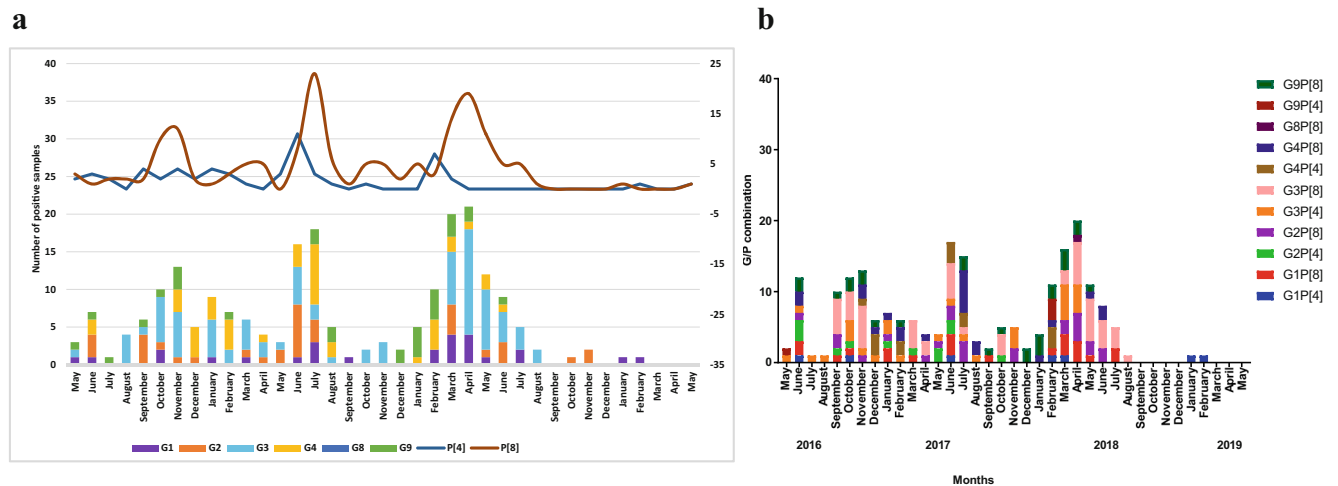


Fig. 5 Annual distribution of RV infections per month in Qatar. **a** Seasonal trends of RV G and P serotypes in Qatar represented as bar chart for G type and represented as line for P type. **b** Seasonality of circulating G/P combination in Qatar represented as bar chart between May 2016 and June 2019

RV genotypes associated with the gender

Generally, we observed higher infections in males compared to females between genders. G3P[8] was the most prevalent strain in both genders, but being higher in males (70%, $n = 50/71$) compared to females (30%, $n = 21/71$). Other G/P combinations G4P[8], G4P[4], G3P[4], G9P[8], G1P[8], and G1P[4] were more prevalent in male children by 74%, 62%, and 64%, compared to female children. On the other hand, genotypes G2P[8] and G9P[4] were detected equally in both male and female genders (Fig. 6a).

RV genotypes associated with the age of children

We next evaluated the association between circulating RV genotypes and the age of patients. The most predominant RV genotype G3P[8] was at least two-fold higher than other genotypes in children < 1 year of age, representing 59% ($n = 42/71$) of the cases (Fig. 6b). In the 1–3-year age group, G1P[8], G2P[8], G4P[4], G3P[4], and G9P[8] genotypes were detected at close frequencies of 15%, 17%, 10.2%, 8.4%, and 7.5% respectively. Generally, a low number of infections were reported in > 3-year-old children, with G3P[8] and G9P[8] being marginally higher than other genotypes at the frequency of 23% and 19.2%, respectively (per that specific group).

RV genotype-associated clinical outcomes

We next evaluated the association of clinical outcomes in hospitalized patients with circulating genotypes using the Vesikari Clinical Severity Scoring System [27] (Fig. 7a). It was interesting to observe that 98% of RV-infected subjects had severe symptoms with a high Vesikari score (score ≥ 10). The severity of RV infections was noticed higher between

both children < 1 year old and 1–3 years of age compared to > 3 years of age. The maximum number of children reporting diarrhea for more than 3 days was detected with G3P[8] (21%, $n = 15/71$) (Fig. 7b). Similarly, the greatest number of children reporting vomiting severity score for more than 3 days was in the G3P[8] group (35%, $n = 15/43$), followed by G9P[8] (14%), G4P[8] (14%), and G1P[8] (14%). Furthermore, dehydration was relatively severe in the G3P[8] (50%, $n = 4/6$), G4P[4] (16.5%, $n = 1/6$), and G2P[4] (16.5%, $n = 1/6$) groups. A high number of bloody loose stools were detected more frequently in subjects with G3P[8] (70%, $n = 7/10$), compared to G2P[8] (20%, 2/10) and G9P[8] (10%, 1/10). Moreover, subjects with high fever between 38 and 40 °C were noticed with G3P[8] (30%, 3/10), G2P[8] (30%, 3/10), G1P[8] (10%, 1/10), G4P[4] (10%, 1/10), G9P[8] (10%, 1/10), and G1P[8] (10%, 1/10). Additionally, subjects rehydrated and hospitalized in PEC for more than 3 days were detected with G3P[8] (29%, 5/17), G4P[8] (29%, 5/17), G2P[4] (18%, 3/17), G2P[8] (18%, 3/17), and G4P[4] (6%, 1/17).

RV genotypes association with nationality

Qatar is a multinational country where expats represent more than 85% of its population. We, therefore, investigated the correlation between circulating RV genotypes and the nationality of patients. The ratio of Qataris admitted was more compared to other nationalities. All dominant genotypes in this study G3P[8] (21:50), G9P[8] (5:22), G2P[8] (5:9), and G1P[8] (7:17) had a higher incidence in Qatari children. Likewise, less dominant genotypes such as G4P[4] (3:4), G3P[4] (7:10), and G2P[4] (7:5) had also higher incidence rate among Qatari children. G4P[8] (6.3%, 5/79) depicted a similar frequency in both Qatari and Pakistani children. Interestingly, G9P[4] was not detected in Qatari children,

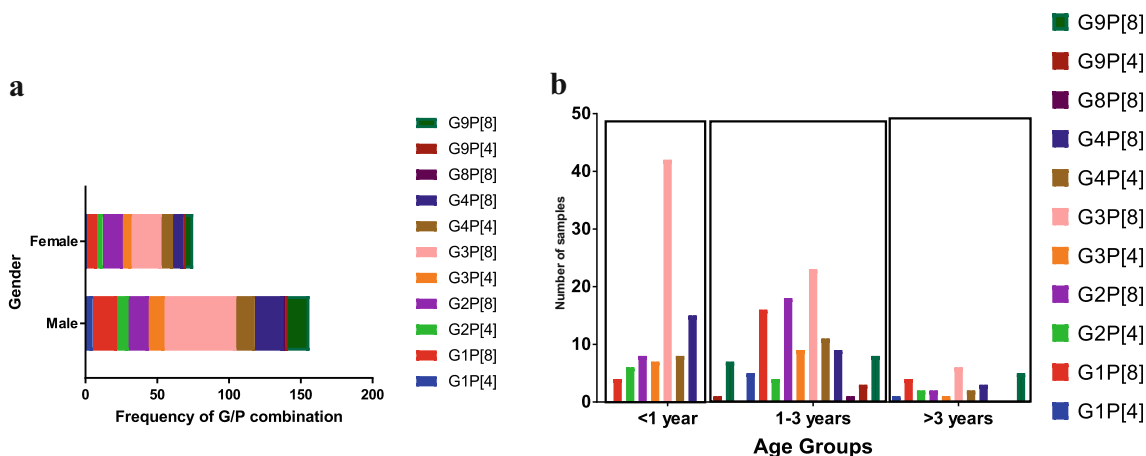


Fig. 6 Prevalence of circulating G/P combination. **a** Frequency of circulating RV genotypes represented as G/P combination in *X* axis associated with gender represented in *Y* axis. **b** Association between genotypes and

age groups of RV-infected children during the study period between May 2016 and June 2019

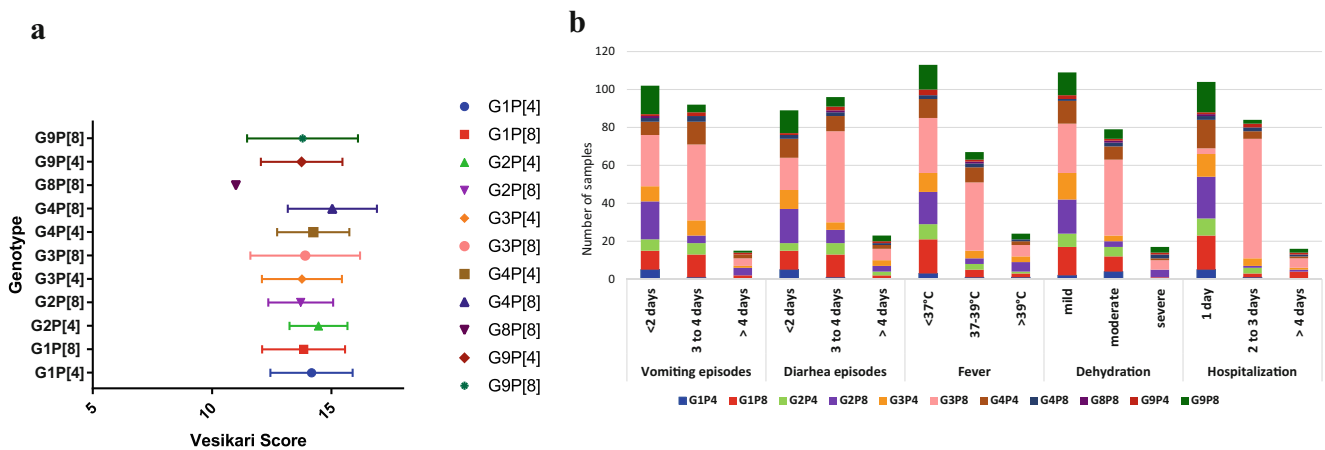


Fig. 7 **a** Association between genotypes and clinical illness in infected children as measured with Vesikari Clinical Severity Scoring System. The circulating genotypes are represented in *Y* axis and the Vesikari score is represented in *X* axis (mild: score < 7, moderate: score 7 <

score < 10, and severe: score > 10). **b** Association between genotypes and episodes of clinical illness observed in RV-infected children. The number of samples is represented in *Y* axis and the episodes of clinical illness are represented in *X* axis

although it had higher incidence rates in children from Saudi Arabia (25%, 1/4), Pakistan (25%, 1/4), Philippines (25%, 1/4), and Egypt (25%, 1/4). The top four populations infected with G3P[8] were from Qatar (29.5%, 21/71), Egypt (11.2%, 8/71), India (11.2%, 8/71), and Pakistan (11.2%, 8/71). Distribution of all genotypes among different nationalities is depicted in Fig. 8.

Discussion

Qatar, one of the smallest nations by size and area in the world, is incredibly populated with diverse nationalities (http://www.fhrc.org/science/labs/hahn/methods/mol_bio_meth/Big%20Dye%20Protocol.pdf). Every day, thousands of expats arrive from every corner of the globe to this tiny country. This may present a threat for introducing several types of pathogens into the country, and henceforth, it necessitates active surveillance programs to understand the epidemiology of these pathogens and prevent their spread locally. This, in fact, becomes more important considering

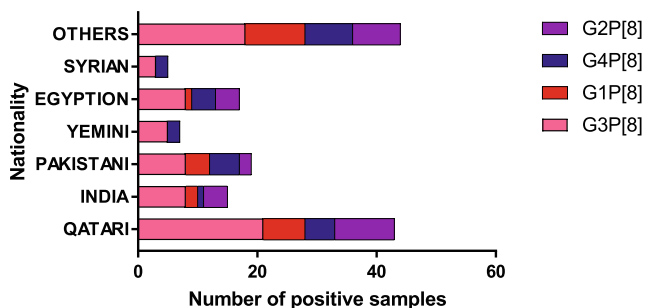


Fig. 8 Bar chart representing the percentage of dominant RV genotypes among top five population during the study period from May 2016 to June 2019

Qatar is preparing and hosting World Cup 2022 and many other mass-gathering public events.

AGE is the second leading cause of children infections worldwide, resulting in at least two million hospitalizations annually [28]. Viruses remain the primary cause of AGE worldwide, specifically RV and norovirus, followed by astrovirus, adenovirus, sapovirus, and others [28]. Little is known about molecular characteristics of circulating viruses and their clinical significance in Qatar. An earlier study in 2013 reported around 10.4% prevalence of the virus among children and adults with AGE illness [21]. However, the study was limited to 1 year and did not investigate the circulating RV genotypes.

In this study, we report on the epidemiology, genotype characterizations and distribution, and disease severity of RV infections among children suffering from AGE in Qatar between May 2016 and June 2019. Several studies from the MENA region had evaluated the different aspects of RV-associated AGE including incidence of RV in hospitalized AGE ($n = 37$ studies), genotype detection and distribution ($n = 25$), disease severity and consequences ($n = 9$), use of health care system ($n = 11$), treatment costs ($n = 2$), and mortality ($n = 1$). The following MENA countries detailed the prevalence rate of RV infection, which includes Egypt [26, 29–31], Iran [30, 32–36], Iraq [30, 37], Bahrain [38], Lebanon [3, 39], Jordan [30, 40], Kuwait [41], Libya [35, 42], Morocco [43], Oman [44, 45], Saudi Arabia [46–49], Syria [30], Tunisia [50–53], Turkey [54–58], the United Arab Emirates [59], Israel [60, 61], and Yemen [30].

Our analysis demonstrates that the rate of RV-associated AGE cases in Qatar was about 28.4% ($n = 231/812$). This is relatively higher than other reports from Egypt (23%) [26, 29, 30, 62], Saudi Arabia (20%) [46–49], and Tunisia (20%) [50–53], but lower than reports from Syria (61%) [30],

Oman (50%) [44, 45], the United Arab Emirates (50%) [59], Kuwait (44%) [41], Bahrain (44.8%) [38], and Lebanon (30.3%). However, there was no significant difference between the percentages of RV infection in the above studies when compared to our study. As for the length of the study, almost all these studies had comparable sample sizes and used similar case definition and molecular assays.

Further, concerning gender, the number of males infected with RV was higher compared to females, with a ratio of 1.4:1. This result is concordant with majority of previous studies from MENA region that includes Bahrain [38], the United Arab Emirates [59], Iraq [63], Iran [64], Bahrain (10), Iraq (22), Jordan (25), Morocco (29), Saudi Arabia [46–49], Tunisia [50–53] Turkey (34, 35), and Yemen (37) and from other countries like Nigeria [65], Kenya [66], Morocco [43], and Ghana [67].

In general, RV infections are mostly reported in children < 1 year of age [68–75]. In this study, 50% of the RV infection occurred in children between 1 and 3 years of age as compared to other age groups (< 1 and > 3 years) [38, 76–78]. Besides, several studies reported that most RV infections occurred in the age group from 12 to 24 months [28, 59, 79–81]. Only one study reported a higher incidence of RV AGE among children aged 1–6 months [82].

In the MENA region, about twenty-one studies from 12 countries reported RV infection in both winter and autumn seasons including Lebanon [39], the United Arab Emirates [59], Bahrain [38], Egypt [26, 29–31], the Islamic Republic of Iran [28, 80, 83–85], Iraq [72], Jordan [77], Libya [69], Morocco [43], Saudi Arabia [68, 86], Tunisia [78], Turkey [81], UAE [59], and Yemen [82]. However, a drop in RV infections was recorded with the onset of colder months in Qatar between September and December (23% of overall cases). The peak of RV infections in our study was noticed only with the onset of warmer months between April and July (spring season). The RV reported cases among children during all study periods represented 48.5% of the overall cases. Such seasonal patterns of RV infection were recorded within the first 2 years of the study, but not the last one, where we observed a near complete drop in RV infections between July 2018 and June 2019, which might be due to lower AGE in general. It is worth mentioning that we did not observe any RV circulation during the winter season in 2018 (September–December). This may be associated with a sudden soar in temperature with extended humidity that was observed during the year 2018. Of note, it has been reported increased cool, dry seasons particularly associated with RV disease which coincides with the winter season because the survival of infective RV is favored in cooler conditions with low relative humidity [87]. It has also been hypothesized that a relative drop in humidity and rainfall combined with the drying of soils might increase the aerial transport of dried, contaminated fecal material. Therefore, in our study, a sudden

soar in temperature with extended humidity during the year 2018 might have caused a decrease in RV infection [88].

We detected six different G genotypes (G1, G2, G3, G4, G8, G9) and two P genotypes (P4, P8) distributed into 11 G-P combinations. G3 (38.5%) was the most predominant G type, followed by G4 (20%), G2 (16.6%), G9 (12.5%), G1 (12%), and G8 (0.4%). Interestingly, a decrease in G3 incidence in certain months was associated with the predominance of G1, G2, and G9. No previous studies demonstrated such observation, so it is not known whether the dominance of G1, G2, and G9 is attributable to natural fluctuations in RV genotypes or if they represent a unique situation in Qatar. Lately, G3, a ubiquitous genotype, has increased its frequency in most of the countries like Pakistan [30], Japan [17, 18], (http://www.fhrc.org/science/labs/hahn/methods/mol_bio_meth/Big%20Dye%20Protocol.pdf), China [19, 40], Vietnam [41], and Hong Kong [23], and has replaced G1, G2, and G9. It has been postulated that heterotypic strains are being selected by vaccination pressure; however, it has not been established whether these observations associated domination of G1, G2, and G9 is a result of a vaccine-induced immunological pressure or reflect natural temporal variations in the circulation of RV genotypes [89].

G3P[8] was the most dominant combination, accounting for 30.8% ($n = 71$) of all combinations. This observation was concordant with minimal studies from the Middle East region, Egypt (2011–2012) [45], Tunisia (2003–2005, 2009–2010) [158] [164], Israel (2007–2008), Iran (2005–2015), Jordan (2007–2011), Kuwait (2007), and Saudi Arabia (2007–2008). Recently, the G3 serotype is recognized as the third most predominant RV genotype in humans, mostly found in combination with P[8] [90]. Although no direct causal link has been proven, G3 strains with different P types P[4], P[6], P[8], and P[9] have been detected in humans around the world after the introduction of RV vaccines in immunization program of many countries. This might be the reason for the evolution of new variant genotypes that emerged as a predominant genotype in Asia, Europe, and South America with high similarities with equine-like G3P[8] strains [91]. G3P[8] strains initially originated in the USA from 1974 to 1991 and found that all the strains belonged to the Wa-like genetic backbone [92].

As observed in the concatenated tree, G3P[8] viral strains in our study detected from 2014 to 2019 were genetically grouped into a single cluster, which was distinct from another cluster comprising G3 strains (cluster 1). Within cluster 1, some of the G3 strains showed similarity close to strains circulated in Italy in 2012–2013 and belonged to equine-like G3P [8] RV strains (GenBank: KT988229.1). Likewise, few G3 strains showed high nucleotide sequence identity close to Wa-like genomic constellation strains that circulated in Eastern India in 2014–2016 (GenBank: MF563894.1) and DS-1-like genomic backbone strain Japan in 2017–2018 (GenBank: LC386081.1). It has been shown that shortly after

Rotarix introduction atypical DS-1-like genomic backbone spread rapidly in the human population compared to Wa-like genomic constellation strains [93–95]. This could be the result of reduced protection afforded by the Wa-like Rotarix vaccine, which provided an opportunity with the emergence of DS-1-like genomic backbone [96]. These findings suggest that the genome of G3 strains might have evolved as a whole chronologically, from modern recently circulating G3 RV and due to higher vaccine effectiveness.

G2P[8] was the second most predominant genotype in our study, reaching 12.3% ($n=28$) overall prevalence. The G2 strain detected in our study was closely related to strains from Pakistan (GenBank: MF673444.1), Turkey (GenBank: MF494809.1), and Lebanon (GenBank: MH591274.1). It was markedly high during the 2016–2018 season, but it was negligible in the rest of the study period. These fluctuations may be due to the average interseasonal diversity of these circulating strains in the region [97]. Around six countries observed the circulation of this genotype as dominant between 2005 and 2008. This includes Egypt (2006–2008), Israel (2007–2008), Kuwait (2005–2006), Morocco (2006–2007), Turkey (2004–2005), and Yemen (2006–2008).

G4P[8] was the third most prevalent genotype and was closely related to the viral strains from Russia (2008) (GenBank: FJ440333.2). Genotype G4P[8] was the predominant etiological agent of RV infection in Russia, responsible for about 52% of RV AGE cases in the country [98]. However, molecular epidemiological data indicate that in the last three decades, genotype G4P[8] played an important role in the European region and other parts of the world as a causative agent of AGE, being responsible for up to 60% of annual cases in various countries [98].

In addition to the above, we investigated the correlation of the circulating genotypes and the nationality of the patients. The most predominant genotypes in our study, which include G3P[8], G4P[8], G2P[8], and G1P[8], were detected with a higher incidence rate in Qatari children compared to other nationalities. The top four nationalities infected with G3P[8] were Qatari (29.5%, 21/71), Egyptians (11.2%, 8/71), Indian (11.2%, 8/71), and Pakistani (11.2%, 8/71). G3P[8] accounted for 15.6% in Egypt [99], and 5.9% in northern India [100] during 2016–2017. Currently, G3P[8] is the predominant genotype circulating in Pakistan (22.4%) [101]. Likewise, G2P[8] genotype detected in our study was more frequently detected among nationalities from Egypt, India, and Pakistan, and it was reported to be the most predominant genotype in the respective countries. Moreover, G4P[8] represented a similar incidence among pediatrics of both Qatari and Pakistani origin. On the other hand, G9P[4] was not noticed in pediatrics of Qatari origin but among Saudi Arabian (25%, 1/4), Pakistani (25%, 1/4), Philippine (25%, 1/4), and Egyptian (25%) pediatrics. Besides, G4P[8] and G1P[8] genotype combinations detected in our study were the most common in MENA countries during 2010–2016 and worldwide. However, RV strain

distribution has been shown to vary greatly among the Middle Eastern countries, for example, in Iran, G4P[8] is the most common RV strain, while a different RV strain distribution was observed in Oman [44]. Indeed, WHO surveillance data across the Eastern Mediterranean region indicated substantial strain diversity in the RV types circulating in this region [59].

We finally evaluated the correlation between circulating genotypes and clinical outcomes using the Vesikari Clinical Severity Scoring System. It was interesting to observe that 98% of our admitted subjects with RV infections had severe symptoms with a high Vesikari score (score ≥ 10). Besides the Vesikari score system, G3P[8] associated with the highest diarrhea and vomiting scores, which was concordant with the study reported by Iturriza-Gomara et al. (2000) who reported that G3P[8]s belong to the most characterized RV strains that can cause severe diarrheal diseases in humans [99].

In conclusion, the results of this study provide the most comprehensive and up-to-date information on the RV circulating strains in Qatar. We detected six different RV G serotypes, two different P serotypes, and eleven different G-P combinations. G3, G9, G2, and P[8] were found to be the predominant genotypes, followed by P[4]. Analysis of the clinical records showed there is a difference in clinical manifestations or severity with genotype. Therefore, continuous surveillance for the RV circulating genotypes and assessment of their clinical significance is warranted, particularly Qatar is preparing for World Cup 2022. The availability of these data will facilitate the detection of future importation of new strains into the country.

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Performed the experiments: SMM
Analyzed the data: SMM, HMY
Wrote the manuscript: SMM, HMY
All authors revised the manuscript and agreed with the final submitted version.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available (still in the process of submission to GenBank) but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval The approval was obtained in compliance with ethical standards.

Consent to participate Informed consent was obtained from legal guardians.

Consent to publish Informed consent were obtained from the patients to publish.

Competing interests The authors declare that they have no conflict of interest.

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