Emergence and high prevalence of unusual rotavirus G8P[8] strains in outpatients with acute gastroenteritis in Shanghai, China

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Abstract

Group A rotavirus (RVA) is considered an important cause of acute gastroenteritis (AGE) in all age groups, especially in children. We investigated the epidemiology of RVA in outpatients aged [?]16 years at the Children's Hospital of Fudan University, Shanghai, China. In this study, 16.6% (246/1482) were infected with RVA. The detection rate of RVA was significantly higher in the year of 2021 (20.3%, 147/725) compared to the year of 2020 (14.5%, 77/531) and 2022 (9.7%, 22/226) (p=0.000). RVA infection was prevalent in all seasons from 2020 to 2022, with a different monthly distribution observed in different years. Among 246 RVA-positive samples, 14 different RVA genotypes were detected with different frequencies. Overall, G9P[8] (45.5%, 112/246) was the most common RVA genotype, followed by G8P[8] (37.4%, 92/246) and G3P[8] (4.1%, 10/246). The prevalence of G/P combinations varied from 2020 to 2022. G9P[8] was the most circulating genotype in 2020 (68.2%, 15/22) and 2021 (57.8%, 85/147). However, G8P[8] (68.8%, 53/77) suddenly became the most prevalent genotype in 2022 after being first identified in 2020 and prevalent in 2021. The G8 strains detected in the study were all clustered to DS-1-like G8 strains with the closest genetic distance to strains circulating in Southeast Asia. Our study demonstrated the diversity of circulating RVA genotypes in Shanghai. The sudden emergence and high prevalence of unusual G8P[8] strains deserve more concern and indicate the need for continuous surveillance of RVA in children with AGE in the future to refine future vaccine strategy.

1 INTRODUCTION

Acute gastroenteritis (AGE) has been identified as the second leading cause of death in children <5 years of age, causing approximately 500,000 deaths annually worldwide [1]. Group A rotavirus (RVA) is the most common cause of AGE in young children especially under five years of age worldwide[2,3]. As the main pathogen of AGE, RVA infection and death occur in all countries worldwide, but mainly in developing countries [4,5]. Based on the Global Burden of Disease 2019 Study (GBD 2019), RVA caused a higher death burden in African, Oceanian, and South Asian countries in the past three decades[6].

Rotavirus was first discovered in 1973 by Bishop and her colleagues in duodenal biopsies of children with AGE [7]. Rotavirus is a non-enveloped RNA virus of the family *Reoviridae* in the subfamily *Sedoreovirinae* , belonging to the genus*Rotavirus* , with a spherical shape and size of 70 nm. It consists of a genome of 11 segments of double-stranded RNA (dsRNA) surrounded by a three-layered icosahedral protein envelope [8]. The genome segments encode six structural viral proteins (VP1-4, VP6 and VP7) and six non-structural proteins (NSP1-5/6). The two outer capsid proteins, VP7 (glycoprotein) and VP4 (protease-sensitive protein) contain neutralizing epitopes and induce protective immunity. The middle layer consists of VP6, while the inner layer is formed by VP2, which encloses two proteins, VP1 and VP3 [5]. Based on the antigenicity of the VP6 protein, ten RV groups (A-J) and two new tentative groups (K and L) have been documented. RVA are by far the most important species in the world in clinical and epidemiological terms[9-12]. RVA is further subdivided into G and P genotypes based on VP7 and VP4, respectively [8]. Currently, about 42 G genotypes and 58 P genotypes have been reported to the Rotavirus Classification Working Group (RCWG)

(https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg)[13]. Among these, several genotype combinations, including G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8], together account for an estimated 90-95% of all RVA infections worldwide[8,14-16]. In contrast to high-income countries, there is a wide diversity of RVA strains in African countries, where atypical RVA, including the G1P[4], G1P[6], G8P[4], G8P[8] and G6P[6] genotypes, are also commonly characterized[8].

Vaccination is considered the primary public health approach to control RVA infection and reduce associated morbidity and mortality[3,17-19]. Currently, two live-attenuated vaccines, monovalent Rotarix and pentavalent RotaTaq, are available for global use. Two Indian-manufactured vaccines, monovalent Rotavac and pentavalent Rotasiil, were recently prequalified by WHO in 2018[20]. In China, two RVA vaccines have been licensed for the Chinese market: Lanzhou lamb RVA vaccine (LLR) consisting of RVA serotype G10P[12] (Lanzhou Institute of Biological Products) licensed in 2001, and RotaTeq developed from the bovine WC3 strain reassorted with human strains G1-G4 and P[8] (Merck) licensed in 2018 [21]. However, most children in China do not receive RVA vaccination in time due to the high additional cost of RVA vaccination [18]. Given the diversity of RVA genotypes and the variety of RVA vaccines, long-term and continuous surveillance of the epidemiology of RVA is essential to provide implications to health authorities on suitable policies for RVA vaccination in children.

In Shanghai, studies on RVA infection in children with AGE during 2020 to 2022 were lacking [22]. To better understand the role of RVA in children younger than 16 years with AGE in Shanghai, we determined the age distribution, gender distribution, seasonal pattern and genotypic distribution of RVA and updated the profiles of RVA genotypes from May 2020 to December 2022.

2 MATERIALS AND METHODS

2.1 Study design

Fecal samples were collected from 1482 children up to 16 years of age who visited the outpatient clinic of the Children's Hospital of Fudan University and were diagnosed with acute gastroenteritis between January 2020 and December 2022 in Shanghai. All enrolled samples were routinely collected and stored at -70°C before the examination. The definition of AGE was three or more loose, watery, thin, pasty stools or the presence of mucous stools within 24 hours, possibly accompanied by vomiting, abdominal pain, fever, and nausea. This definition excluded the presence of pus or blood regardless of the presence of fever[22]. Demographic information and clinical diagnoses were obtained from the patients' medical history. The study proposal was approved by the Institutional Review Board of the Children's Hospital of Fudan University. All procedures were performed in accordance with relevant guidelines and regulations.

2.2 RVA detection

Viral RNA was automatically extracted from 200 μL supernatant stool samples using the Nucleic Acid Extraction Kit (Tianlong Biotechnology). Two-step reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the presence of RVA in each stool sample, cDNA was synthesized with a random primer using Prime-Script II Reverse Transcriptase (Takara Biotechnology) with the reaction condition:97°C 5 min, 37°C for 30 min, 85°C for 5 sec and hold at 4°C. After that, 384 bp of the VP6 gene was then amplified using previously published primers[23]. The amplification condition was 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and with a final extension at 72°C for 5 min.

2.3 G and P Genotyping

Each RVA-positive stool specimen confirmed by sequencing was performed with the semi-nested multiplex RT-PCR to detect six G genotypes and six P genotypes with primers shown in table 1. A detailed explanation is as follows: first round PCR reaction for G genotypes was amplified with primers of VP7-forward (F) and VP7-reversed (R) [24]; and then G genotypes was performed with 2 μl first round PCR product and a mixture of 20 mmol of each specific primer for G1-G4, G8, G9 and VP7-R primer with the length of amplified products being 618, 521, 682, 452, 754 and 179 bp, respectively [25]. Similarly, the first-round PCR reaction for P genotypes was conducted with primers of VP4-forward and VP4-reversed. P genotyping was performed

with 2 μl of first-round PCR product and a mixture of 20 mmol each of internal primers for P[4], P[6], P[8], P[9], P[10], P[11], and VP4-F primer, with the length of the amplified products being 362, 146, 224, 270, 462, and 191 bp, respectively[26]. Thermal cycling of G or P genotypes was performed as follows: 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 44°C for 30 sec, 72°C for 30 sec, and with a final extension at 72°C for 5 min. All amplified PCR products were electrophoresed on 2.0% agarose gels containing Super GelBlue (Shanghai BioScience) and visualized using an automated gel image analysis system (Shanghai Tanon Life Science).

All RVA G8 genotype amplicons were purified and sequenced for phylogenetic analysis using first-generation sequencing technologies (Shanghai Sangon Biotech). Phylogenetic trees were constructed using the maximum-likelihood method (Kimura two-parameter substitution model with 1000 bootstrap replicates for branch support) in MEGA (v6.0) software. The nucleotide sequences of the G8 genotype detected in this study were compared with the sequences of corresponding reference strains available in the GenBank database. The nucleotide sequences of the RVA strains and the accession numbers used were as follows: G1: HM560972; FJ348346; GU183204; EY033973. G2: U73941; KC152925; FJ460820. G3: FJ460831. G4: FJ386453; EF011974; GU183208. G4: FJ386453; EF011974; GU183208. G8: LC133530; LC169879; LC169956; LC169967; LC386070; MG996097; MN058740; MT410500; GU984761; D01054; AB749175; DQ005109; DQ005120; KJ748481; KP883176; KX632259; KX655456; KX655467; LC406824; MG181903; MK434791; MN921185; DQ995179; EF218677; JQ988904; MT232573; FJ611782; JN831225; KJ411440; MF940612; MG214342. G9: MW254156; MW384390; LC477377.

2.4 Statistical analysis

The difference in RVA detection rates, proportions of categorical variables in each group was compared using a two-sided chi-square test in SPSS Statistics v.19.0 (IBM Corp., Armonk, NY, USA), and ap -value less than 0.05 was considered statistically significant.

3 RESULTS

3.1 Prevalence of RVA infections in children

During the study period, a total of 1482 stool samples from outpatient children diagnosed with AGE were included in our study, of which 875 were boys and 607 were girls. Overall, 16.6% (246/1482) of children were infected with RVA. A slightly higher detection rate was observed in 2022 (14.5%, 77/531) compared to 2020 (9.7%, 22/226), but without statistical significance (p =0.078). In contrast, the detection rate of RVA was significantly higher in 2021 (20.3%, 147/725) compared to that in 2020 and 2022 (p =0.000). Furthermore, a similar frequency of RVA was observed in both males (16.6%, 145/875) and females (16.6%, 101/607) (p =1.000).

3.2 Seasonal and age distribution of RVA-infected children

Although RVA infection was prevalent in all seasons from May 2020 to December 2022, a different monthly distribution was observed in different years. The infection rate of RVA increased rapidly and peaked in September in 2020 (41.7%, 10/24). In 2021, RVA infection was detected mainly in winter and spring, peaking in March (37.9%, 11/29) and May (37.8%, 48/127). In 2022, an unexpectedly high detection rate of RVA was observed in February (71.0%, 22/31) and March (68.4, 13/19) (Figure 1). Age group analysis of RVA-positive cases showed that RVA can infect children of all ages. Similar RVA detection rates were seen in children aged 0-72 months (16.3%, 207/1273) and older children aged from 73 months to 16 years (18.7%, 39/209) (p =0.422). The detection rate of RVA-positive cases was highest in children aged from 25 to 36 months (18.9%, 27/143), followed by children over 73 months (18.7%, 39/209). The lowest detection rate was observed in children aged 0-12 months (14.2%, 78/551) (Figure 2). Of the 246 children infected with RVA, 64.6% (159/246) were aged 0-36 months.

3.3 Distribution of G Genotype of RVA

In this study, VP7 region analysis of 246 RAV-positive specimens revealed a large genetic diversity of cir-

culating RAV strains. Except for 16 RVA-positive samples that could not be successfully genotyped, seven genotypes were identified during the study period, including G1, G2, G3, G4, G6, G8, and G9. Among them, G9 (46.3%, 114/246) was the most common G genotype, followed by G8 (37.4%, 92/246) and G3 (4.1%, 10/246). The prevalence of G genotypes varied over the years. Only three and four definite G genotypes were identified in 2020 and 2022, whereas seven definite G genotypes were found in 2021. G9 was the predominant genotype in 2020 (68.2%, 15/22) and 2021 (59.2%, 87/147). In contrast, G8 (68.8%, 53/77) suddenly became the most popular genotype in 2022, after being first identified in 2020 and prevalent in 2021. The second most common G genotype was G8 (25.9%, 38/147) in 2021, while G9 (15.6%, 12/77) shifted to become the second most common genotype in 2022(Figure 3A).

3.4 Distribution of P Genotype of RVA

Among 246 RVA-positive samples, 245 samples were successfully genotyped based on the partial VP4 region. Considering the distribution of P genotypes, four P genotypes (P[4], P[6], P[8], and P[10]) were identified from 2020 to 2022. P[8] remained the undisputed most prevalent genotype circulating in Shanghai, with proportions of 95.5% (21/22), 95.9% (141/147), and 100.0% (77/77) from 2020 to 2022, respectively. In addition, the other three P genotypes (P[4], P[6], and P[10]) were rarely present only in 2021(Figure 3B).

3.5 Distribution ofG/P Genotype of RVA

Regarding G/P combinations, 13 different RVA genotypes were detected with different frequencies in this study. Overall, G9P[8] (45.5%, 112/246) was the most predominant RVA strain, followed by G8P[8] (37.4%, 92/246) and G3P[8] (4.1%, 10/246). Some other uncommon strains such as G1P[8], G1P[10], G2P[8], G4P[8], G6P[8], G9P[4], and G9P[6] were detected very rarely and accounted for a total of 6.1% (15/246) of the total genotyped strains. Notably, partially genotyped and un-genotyped RVA strains were detected at a low frequency (6.9%, 17/246).

However, the circulation of G/P combinations varied from 2020 to 2022. G9P[8] was the most circulating genotype in 2020 (68.2%, 15/22) and 2021 (57.8%, 85/147), while G8P[8] (68.8%, 53/77) emerged as the most predominant RVA strain in 2022. It is noteworthy that G8P[8] was identified for the first time in 2020 and showed a marked epidemic as the second most prevalent RVA strain in 2021 (25.9%, 38/147). On the contrary, the proportion of G9P[8] strains, which were predominant in 2020 and 2021, showed a sharp decrease in 2022 (15.6%, 12/77). The second most common genotype in 2020 was G-P[8] (18.2%, 4/22). In addition, G3P[8], which was identified as the third predominant RVA strain in this study, was mainly detected in 2021 (90.0%, 9/10). (Figure 3C).

3.6 Gender and ages distribution of RVA strains

There were 12 and seven RVA strains detected in male and female children, respectively. The predominant RVA strains detected in males were the same as in females. Regarding the age distribution of RVA strains, the main prevalent genotypes (G9P[8], G8P[8] and G3P[8]) were detected in almost all age groups. However, all the RVA genotypes identified in this study were circulating in children aged 0-72 months, while only four RVA strains were detected in children older than 73 months. To our surprise, G8P[8] was the most common genotype in children aged >73 months (59.0%, 23/39), while the frequency of G8P[8] in children aged 0-72 months was 33.3% (69/207) (Table 2). Furthermore, the proportion of the G8P[8] strain in these two age groups was statistically significant (p =0.004).

3.7 Sequence analysis of VP7 segments deserved to G8 genotype

To better understand trends in the epidemiology of G8 strains, a representative number of samples were included in the sequence and phylogenetic analysis. In our study, 72 out of 92 strains were successfully sequenced and the VP7 sequence was 881 bp in length. According to the phylogenetic analyses, all 72 VP7 nucleotide sequences of G8 were clustered exclusively in lineage 1 of DS-1-like G8 strains previously isolated from several Asian regions (Figure 4). Further analysis showed that the VP7 nucleotide sequence similarities among themselves were 89.2%-100.0%. The Shanghai strains were most closely to the reference strains isolated from other Asian regions in nucleotide sequences identity (94.1% to 99.6%), while the nucleotide sequence

similarity between the Shanghai strains and the references reported from other regions was relatively low with the nucleotide sequence identity being less than 90.0%. Of note, the nucleotide sequence identities between the Shanghai strains and bovine strains ranged from 72.9% to 96.2%, while the amino acid sequence among them showed 88.5%-96.8% sequence identity with each other.

4 DISCUSSION

RVA is still the most common cause of non-bacterial AGE in children in China[27,28]. The current study provides useful data in the context of RVA infection and strain diversity in children [?]16 years of age in Shanghai, highlighting the importance of routine surveillance for the changing predominant strains to guide relevant vaccination activities.

In this study, we closely monitored the trend of RVA infection in Shanghai from May 2020 to December 2022. According to the available data, RVA was still an important pathogen inducing AGE in Shanghai, with the average detection rate of RVA being 16.6% during the study period. The prevalence of RVA infection in children with AGE was similar to the data from our previous study during 2012 and 2018 (13.6%)[22]. All these data suggested that the burden of AGE attributed to RVA among outpatient children in Shanghai did not change much during these years. Although LLR vaccine has been available in the private market since 2000, vaccination coverage among children in Shanghai is still low [29]. Considering that the children enrolled in this study were outpatients with mild symptoms and the LLR vaccine showed high efficacy against severe and hospitalized AGE[20,30,31], therefore, immune protection induced by RVA vaccine may not yet be demonstrated in outpatients with AGE. In China, recent large-scale national surveillance data showed that the prevalence of RVA was 40.7%, 31.3%, and 11.2% in outpatient children in lower-middle-income, upper-middle-income, and high-income regions, respectively [32,33]. The inclusion of appropriate RVA vaccines in the non-pharmaceutical interventions (NPIs) should be seriously considered as the burden of RVA-related AGE in children in China is still serious.

Interestingly, the detection rate of RVA in 2021 (20.3%) was significantly higher than that in 2020 (9.7%) and 2022 (14.5%). As we all know, the coronavirus disease 2019 (COVID-19) epidemic spread rapidly in China from the end of 2019, and the first COVID-19 case was reported in Shanghai on January 20, 2020. Since then, different level of NPIs (wearing masks, cordoning off, closing schools and day-care centers, ensuring physical distance from others and washing hands) were implemented in Shanghai until December 2022. Some studies also found that the NPIs have a significant impact on the spread of many infectious diseases, including gastroenteritis viruses [34-36]. This significant reduction in RVA infection observed in Shanghai in 2020 and 2022 maybe influenced by the strict implementation of the NPI strategy. In contrast, the rebounded detection rate of RVA in 2021 may be related to the relaxed implementation of the NPIs strategy because of the low prevalence of COVID-19 in Shanghai during this period.

The temporal distribution of RVA infection among children with AGE in Shanghai has a clear seasonal pattern, mainly concentrated in autumn and winter, which was similar to the seasonal transmission pattern of RVA in most regions of China [22]. However, the analysis of the monthly positive rate did not show a clear seasonal variation of RVA infection influenced by the COVID-19 epidemic during the study period. A relatively high RVA incidence was mainly concentrated in autumn in 2020, winter and spring in 2020-2021 and 2021-2022. In addition, we also found that the prevalence of RVA was at a low level in the winter of 2022. However, in Japan, the peak infection of RVA has gradually shifted from winter to spring since 1981, which may be influenced by warm weather[37]. Hence, continuous monitoring of the seasonal epidemiologic characteristics of RVA in Shanghai in the future is extremely important to identify the true cause of seasonal changes in RVA prevalence. Similar to studies in different regions in China, the prevalence of RVA in Shanghai was mainly concentrated in children aged from 0-36 months [22]. Meanwhile, we found that children younger than 72 months and older children from 73 months to 16 years had similar detection rates. We suspected this phenomenon may be related to the decline in antibodies to RVA with age, which has been demonstrated by several investigators [38-40].

In this study, the most common G genotypes of RVA in worldwide are G1, G2, G3, G4, G9, and G12 [8].

Previous studies of children infected with RVA in China showed that G3 and G1 were the most common G genotypes before 2010, and G9 became a robust common G genotype in children with acute diarrhea in China since 2011[41]. Although G9 was still the most common G genotype among the seven definite G genotypes, the proportion of this genotype showed a significant decreasing trend from 2020 to 2022 and changed to the second common G genotype in 2022. Surprisingly, G8 first appeared in 2020, but it eventually replaced G9 to become the most common genotype in children with AGE in 2022. G8 is one of the more common RVA strains of bovine origin and was first detected in humans in Indonesia between 1979 and 1981 [42,43]. Although it has been detected in children in many countries and has become one of the dominant strains in some sub-Saharan African countries, it was rare in China[21,44]. However, data from a study in Guangzhou showed that a high proportion of infants with severe AGE were infected with G8 RVA recently [41]. Altogether, the data indicate that G8 will be likely replace G9 as the predominant G genotype among RVA-infected children with AGE in China. This hypothesis needs to be supported by more comprehensive epidemiological data on RVA in different regions of China.

Compared with the prevalent G genotypes in this study, only four P genotypes (P[4], P[6], P[8], and P[10]) were detected. Consistent with many other studies worldwide in recent years, P[8] (97.2%) was the absolute most prevalent genotype in Shanghai from 2020 to 2022 [8]. The other three P genotypes were prevalent at low levels and were only detected in 2021.

In this study, 13 RVA genotypes were identified in children with AGE. Differences in the predominance of RVA strains and emerging strains were observed. The circulation of G9P[8] strain from 2020 to 2021 was overall predominant and similar to the rest of the world and our previous data [22]. Interestingly, the G8P[8] unexpectedly became the most prevalent in 2022 with the proportion of 68.8%, which emerged in 2020. To our knowledge, this is the first time that an epidemic of G8P[8] was found in Shanghai as early as 2020[22,41]. Given that two licensed RVA vaccines have been introduced in Shanghai, whether this change is due to the natural immune pressure mechanisms or the RVA vaccination pressure is still unknown[45]. While universal RVA vaccination has progressed worldwide, its impact on the emergence of escape mutants or even the more efficient spread of previously known unusual strains due to selective pressure is still a concern [37,46,47]. Some studies have reported a shift in the proportion of dominant RVA genotypes before and after the introduction of the RVA vaccine[37,46,48]. However, continuous monitoring of RVA genotypes in the pre- and post-vaccine era is essential to refine future vaccine strategy and to understand the true nature of the immune pressure exerted by RVA vaccines against other circulating wild-type strains. Considering that the vaccine efficacy of LLR and RV5 against AGE caused by G8P[8] is still unclear in China, the efficacy and immunogenicity of these vaccines in Chinese children were urgently needed to be clarified.

The circulation of G3P[8], which were the main epidemic strains in Shanghai before 2011, showed a significant decrease during this study[22]. In addition, some other rare RVA genotypes were identified during this study, including G1P[8], G1P[10], G2P[8], G4P[8], G6P[8], G9P[4], and G9P[6] strains. Although 7.0% of RVA samples were inconclusive for G or P genotypes, this was much lower than our previous study (nearly 30.0%)[22]. We have improved the detection method to identify G or P genotype in this study, which may be the main reason. Data from this study imply that appropriate monitoring methods for RVA are essential to find relatively comprehensive prevalent genotypes.

Additionally, phylogenetic analysis revealed that VP7 nucleotide sequences of G8 strains detected in the study clustered together with DS-8-like G8 strains isolated from Japan, Singapore, Thailand, and Korea and shared high nucleotide sequence identity. We also found that the Shanghai strains and bovine G8 strains shared 88.5%-96.8% aa sequence identity with each other. Although some research had confirmed that the G8P[8] strains in Southeast Asia were generated by reassortment of bovine G8 strains and human DS-1-like strains occurred between 2007 and 2012 [49], considering that RVA has 11 gene fragments and we only analyzed the VP7 nucleotide sequence of G8 in this study, we cannot yet conclude that the G8P[8] strains originated from reassortment events between bovine and human strains. Therefore, more and detailed whole genome sequence analysis is needed to clarify the origin of the G8P[8] strain prevalent in Shanghai.

We also analyzed the distribution of RVA genotypes among children in different age groups. We found that

the greatest diversity of RVA genotypes was found in children aged 0-72 months. This phenomenon suggests that children aged 0-72 months are susceptible to multiple genotypes of RVA because the immune response to RVA is not well developed in this age group. Notably, G8P[8] was significantly more common in children aged 73 months to 16 years than in children aged 0 to 72 months. This may also be one of the reasons for the higher detection rate of RVA infection in this age group that we found in this study. Our research suggested that there may be a difference in the age distribution of G8P[8] infection, with prevalence mainly in older age groups. However, this phenomenon still needs to be clarified according to much more detailed epidemiologic data on RVA in people of different ages.

In conclusion, the present study demonstrated the rich diversity of RVA strains circulating in Shanghai. We reported for the first time that the G8P[8] strain emerged in 2020, replacing G9P[8] as the predominant cause of AGE in children with AGE in Shanghai in 2022. Furthermore, the G8P[8] strain was more common in older children than in younger children. Therefore, it is important to continue monitoring RVA genotypes to inform future vaccine strategy to maintain the success and effectiveness of the RVA vaccination program in Shanghai.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTION

Jin Xu designed and supervised the study. Huaqing Zhong, Lijuan Lu, Ran Jia, and Menghua Xu collected the samples enrolled in this study. Huaqing Zhong and Lijuan Lu conducted the experiment and data analysis. Pengcheng Liu, Xuhua Zhu, Liyun Su, and Lingfeng Cao contributed to data collection. Huaqing Zhong and Lijuan Lu analyzed the data and drafted the manuscript. All authors contributed to revising, reading, and approving the submitted version.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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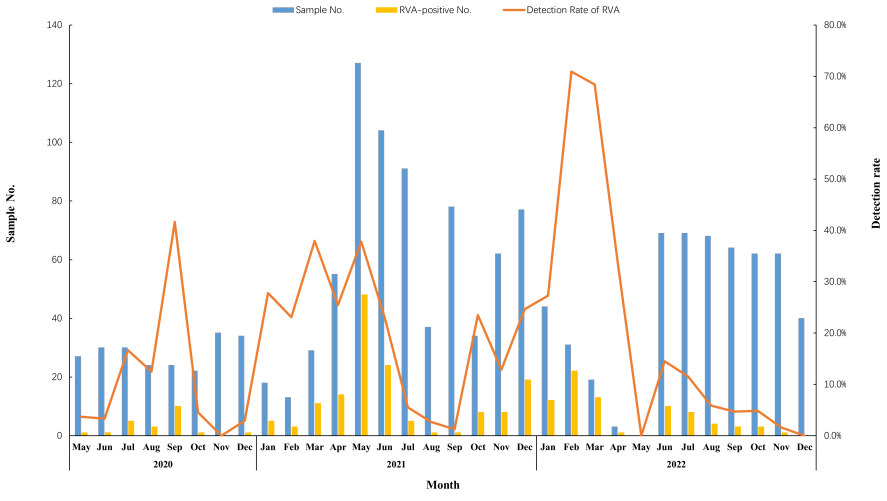
Figure legends

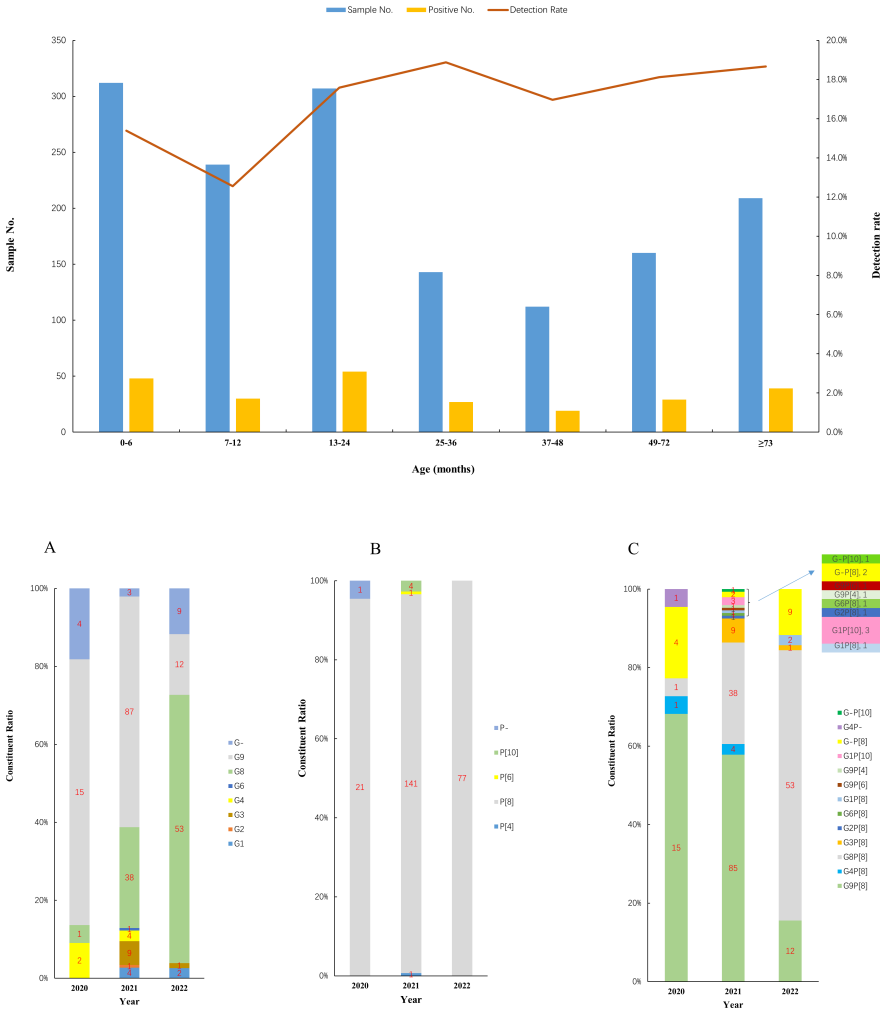
Figure 1. Seasonal distribution of RVA infection in children with acute gastroenteritis in Shanghai, 2020-2022.

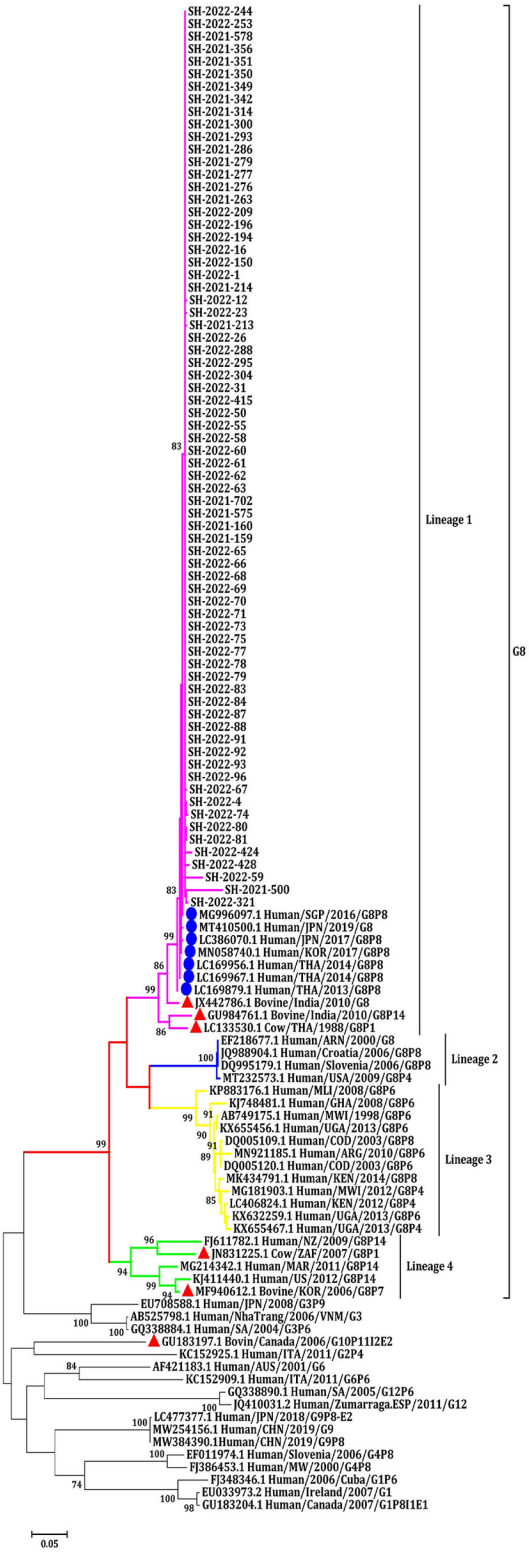
Figure 2. Age distribution of RVA infection in children with acute gastroenteritis in Shanghai, 2020-2022.

Figure 3. Distribution of RVA genotype in children with acute gastroenteritis in Shanghai, 2020-2022. (A) Distribution of G genotype of RVA. (B) Distribution of P genotype of RVA. (C) Distribution of G/P genotype of RVA.

Figure 4. Phylogenetic analysis of the VP7 gene of G8 rotavirus strains. This tree involves 8 different VP7 genes including G1, G2, G3, G4, G6, G8, G9 and G12. The G8 gene was further clustered into four lineages. Studied strains were marked in different shapes and colors. : G8P[8] strains in various regions; : animal-derived strains. The G8 strains detected in this study are indicated by purple lines. References RVA genotypes are labelled according to GenBank with their respective accession numbers. The trees were constructed in MEGA 6.0 through the maximum likelihood method using the Kimura 2-parameter model. The bootstrap values (1000 replicates) are indicated in the phylogenetic tree, and values less than 70% are not represented.







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