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Prevalence and genotype distribution of norovirus in Ningxia Hui Autonomous Region, China, from 2011 to 2022

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Abstract

The norovirus (NoV) genome is diverse. Therefore, this study explored the epidemiological characteristics and genetic features of NoV in Ningxia Hui Autonomous Region, China, from 2011 to 2022 to clarify the genetic diversity in this region. Stool samples were screened for NoV and then sequenced and genotyped. In total, 1,788 of 13,083 specimens were NoV -positive (13.67%); 204 (1.56%) and 1,584 (12.11%) cases were GI and GII, respectively. Additionally, 559 were NoV infection with other viruses (4.27%), primarily with rotavirus (277/559, 49.55%). The NoV incidence rate was the highest among children aged 0–2 years (18.09%, 1054/5,828) and lowest among adults aged 45–64 years (110/1,495, 7.36%); it was also higher in the winter and spring than in the other seasons. GI.3[P3] was the dominant GI genotype. The dominant GII genotype changed roughly every two years. In the GII group, GII.4 was the most common genotype (46.79%), followed by GII.3 (21.34%), GII.2 (12.34%), and GII.17 (9.77%). There were three variants of GII.4 Den Haag, GII.4 New Orleans and GII.4 Sydney identified in the detected GII.4 strains, with GII.4 Sydney dominating. The GII.4 (87.36%), GII.3 (86.35%), and GII.2 (72.92%) strains were primarily detected in children, whereas it was the GII.17 (52.63%) strain in adults. Overall, the NoV genotypes in the Ningxia Hui Autonomous Region were diverse. Primarily, GII groups were dominant, but this changed over time.

Keywords Infectious diarrhea, Norovirus, Epidemiological characteristics, Genotyping

Introduction

Globally, norovirus (NoV) is the leading cause of foodborne illness after rotavirus [1], with a higher burden of disease than other foodborne diseases (Hashemi, Salayani, Afshari, Kafil, & Noori [2], Pires et al., [3]. Uneven economic development has resulted in uneven global research on NoV, with prevalence and incidence rates varying widely among regions and countries (Ogunsakin, Ebenezer, & Ginindza [4]), . Infectious diarrhea causes a considerable number of child deaths and malnutrition each year in Africa, with NoV deaths in children aged below 5 years accounting for approximately 40% of annual global NoV deaths [5]. In the United States and high-income countries in Europe, NoV outbreaks often

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occur in healthcare facilities or nursing homes, exacerbating the condition of patients or causing death in frail populations [6].

NoVs belong to the family Caliciviridae and are single-stranded, positive-sense RNA viruses. The full genome length is 7.5 kb, containing three open reading frames (ORFs): ORF1 to 3 (Lucero, Matson, Ashkenazi, George, & O'Ryan [7]), . Early studies divided NoV into 10 genogroups (GI-GX) based on the amino acid sequence of the capsid (VP1) region, of which GI, GII, GVIII, and GIX predominantly cause human infections. Among these, GII is the most common in clinical surveillance studies worldwide [8].

NoV is susceptible to mutation and recombination in or near the overlapping regions of RNA-dependent RNA polymerase (RdRp) and VP1; therefore, a dual-typing classification system based on these two regions has been recommended internationally since 2019 [8]. Using the new classification, the NoV VP1 region comprises 12 groups containing 48 identified genotypes, of which 9 and 26 belong to the GI and GII groups, respectively. Similarly, the RdRp region genes now comprise 10 groups containing 60 genotypes, of which 14 and 37 belong to the GI and GII groups, respectively. Although many NoV types exist, GII and some GI groups are the primary causes of acute gastroenteritis in humans (Kendra, Tohma, & Parra [9]), . NoV vaccine development is challenging owing to its diverse genome. Although some vaccine candidates are being studied, none are ready for clinical use (M. Zhang, Fu, & Hu [10]), . Understanding the molecular epidemiology of the NoV genotypes is crucial for vaccine development.

The incidence rate and case reports of infectious diarrhea in Ningxia region from 2011 to 2022 were as follows: 132.16/100,000 (8328 cases), 140.88/100,000 (9007 cases), 137.04/100,000 (8869 cases), 125.68/100,000 (8222 cases), 129.73/100,000 (129.73/100,000 cases), 136.67/100,000 (9128 cases), 149.25/100,000 (10,073 cases), 158.94/100,000 (10,836 cases), 118.15/100,000 (8,125 cases), 98.25/100,000 (6,825 cases), 88.13/100,000 (6,348 cases) and 65.17/100,000 (4,725 cases), Although the incidence of infectious diarrhea has been decreasing in Ningxia in recent years, it is still a public health problem affecting local residents. During the study period, by monitoring infectious diarrhea and foodborne diseases and studying the spectrum of viral diarrhea in Ningxia, it was determined that norovirus emerged as the primary pathogen responsible for both sporadic cases and outbreaks in Ningxia. The positive rate of norovirus was found to be 18.73%, making it a significant contributor to infectious diarrhea among infants and elderly individuals. Subsequently, we started a series of investigations were conducted to elucidate the epidemiology and genetic evolution of norovirus.

Therefore, in order to understand the infection status of norovirus in the diarrheal population in Ningxia, this study collected specimens and case information of diarrheal patients in the region from 2011 to 2022, performing nucleic acid detection and sequencing typing on fecal specimens, with the aim of exploring the epidemiological characteristics and genetic features of the virus, as well as providing data reference for the prevention, control, and vaccine research and development of NoV.

Materials and methods

Sample collection

In total, Ningxia Hui Autonomous Region has 15 comprehensive, sentinel hospitals for the active surveillance of foodborne diseases. Fecal samples were collected from patients of all ages with acute diarrhea, a defecation frequency of ≥3 with changing shape (e.g., watery stool), or vomiting (Wilber, Baker, Rebolledo, & McAdam [11]), within 24 h after presentation to one of the sentinel surveillance hospitals from January 2011 to December 2022. Clinical data and personal information were also obtained for each patient. All patients provided informed consent for fecal sample and data collection. All collected specimens were immediately analyzed or frozen at −80 °C until further use.

Sample processing and detection

Phosphate-buffered saline was added to 2 g of fecal specimen, followed by shaking in a vortex shaker for 15 min to create a 10% fecal suspension, and then centrifugation at 3000 rpm for 20 min. Next, 200 μL of the supernatant was taken for viral RNA/DNA extraction via the magnetic bead method using a kit (Bioperfectus Technologies Co., Ltd, Jiangsu, China). Then, the Norovirus GI/GII, Rotavirus, Sapovirus, Astrovirus and Adenovirus Nucleic Acid test kits from BioPerfectus technologies were used to detect the respective viruses by real-time reverse-transcription polymerase chain reaction (RT-PCR). Detection and result interpretation were performed per the manufacturer's instructions.

NoV genotypes analysis

NoV-positive samples with cycle threshold (Ct) value of ≤30 were selected for viral gene fragment amplification, including the ORF1/ORF2 overlap region, using one-step RT-PCR with primers MON432/G1SKR for GI norovirus (579 bp) and MON431/G2SKR for GII norovirus (570 bp) [12]. The optimized amplification procedure was 45 °C for 30 min, 94 °C for 1 min, followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 90 s, and a final incubation at 72 °C for 10 min [13]. The PCR products were observed via 1.5% agarose gel electrophoresis, and the successfully amplified positive products were sent to Kuntairui (Wuhan) Bio Company for sequencing.

Table 1 Norovirus positivity among patients with acute diarrhea in Ningxia Hui Autonomous Region, China, from 2011 to 2022

Year	Cases	NoV infection Cases		Total		χ^2	*P*
		GI	GII	NoV infection Cases	Positive Rates(%)		
2011	690	22	81	103	14.92	79.929	< 0.001
2012	866	25	89	114	13.16		
2013	704	23	91	114	16.19		
2014	725	20	104	124	17.1		
2015	861	25	93	118	13.7		
2016	1120	15	121	136	12.14		
2017	1241	5	229	234	18.86		
2018	1127	1	148	149	13.22		
2019	1250	19	115	134	10.72		
2020	1108	13	170	183	16.52		
2021	1891	25	171	196	10.36		
2022	1500	11	172	183	12.2		
Total	13,083	204	1584	1788	13.67		

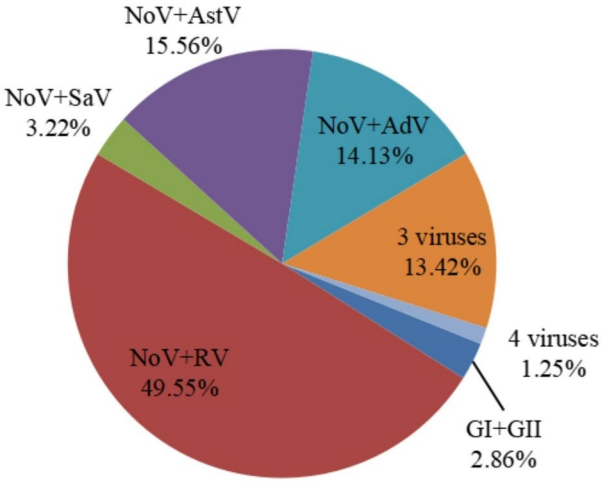


Fig. 1 Co-infection patterns of acute diarrhea cases in Ningxia Hui Autonomous Region from January 2011 to December 2022. AdV: Adenovirus; AstV: Astrovirus; G: Genome; NoV: Norovirus; RV: Rotavirus; SaV: Sappovirus

The feedback sequences were spliced and edited with Sequencher 4.1.4, the NoV typing website (http://www.rivm.nl/mpf/NoV/typingtool), and the National Center for Biotechnology Information website to identify the genotypes [14]. Phylogenetic trees were constructed using the neighbor-joining Mega-X software (Bootstrap 1000×).

Statistical analyses

The database was constructed using Excel 2010 (Microsoft Corp., Redmond, WA, USA) and statistically analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA), using the chi -square test or Fisher's exact test for count data. Statistical significance was set at *P*-vales of <0.05.

Results

Epidemiological features of NoV

From 2011 to 2022, 13,083 fecal specimens were collected from patients with acute diarrhea, of which 1,788 GI/GII NoV-positive samples were identified (detection rate: 13.67%). The predominant group was GII (Table 1). The NoV-positive detection rates significantly differed among the years ($P<0.001$). NoV-positivity varied annually; the highest positive detection rate was in 2017 (18.86%), and the lowest rates were in 2021 (10.36%) and 2019 (10.72%). During the other years, the prevalence was higher in alternate years. Among the positive cases, 559 patients were infected with NoV and another virus (4.27%), most commonly with rotavirus (49.55%, 277/559), followed by astrovirus (15.56%, 87/559) and adenovirus (14.13%, 79/559), as well as all three (13.42%, 75/559) (Fig. 1). The detail of co-infection in norovirus and other diarrhea-causing viruses (Including Adenovirus, Astrovirus, Rotavirus and **Sappovirus**) is shown in Table 2 (differentiation by year).

AdV: Adenovirus; AstV: Astrovirus; NoV: Norovirus; RV: Rotavirus; SaV: Sappovirus; 3 viruses: Any three of the above five viruses were detected; 4 viruses: Any four of the above five viruses were detected.

Population distribution

Norovirus was detected in 14.01% (1,034/7,380) of males and 13.22% (754/5,730) of females, but the detection rate did not differ between the sexes ($P=0.192$). The NoV detection rate was highest among children aged 0–2 years (1,054/5,828, 18.09%), followed by children aged 3–5 years (192/1,242, 15.46%) and adults aged ≥ 65 years

Table 2 Positive detection of Norovirus in diarrhea cases in Ningxia from 2011to 2022, China

year	Cases	Cases of NoV infection								
		GI	GII	Co-infection						
				GI + GII	NoV + RV	NoV + SaV	NoV + AstV	NoV + AdV	3 viruses	4 viruses
2011	690	22	81	0	0	0	2	10	1	0
2012	866	25	89	0	4	0	5	10	4	0
2013	704	23	91	1	9	0	6	3	3	0
2014	725	20	104	0	10	0	5	7	5	0
2015	861	25	93	7	7	1	9	0	6	0
2016	1120	15	121	2	13	3	18	0	9	1
2017	1241	5	229	1	32	4	8	22	18	4
2018	1127	1	148	0	27	2	3	5	3	0
2019	1250	19	115	1	10	3	6	8	3	1
2020	1108	13	170	1	44	2	19	10	11	1
2021	1891	25	171	2	57	1	5	4	9	0
2022	1500	11	172	1	64	2	1	0	3	0
Total	13,083	204	1584	16	277	18	87	79	75	7

Table 3 Sex and age distributions of norovirus-induced acute diarrhea in Ningxia Hui Autonomous Region, China, from 2011 to 2022

Characteristic	Groups	Cases	NoV infection Cases	Total			χ^2	*P*
			GI	GII	NoV infection Cases	Positive Rates(%)		
Sex								
	Male	7380	111	923	1034	14.01	1.700	0.192
	Female	5703	93	661	754	13.22		
Age								
	0–2	5828	105	949	1054	18.09	215.904	< 0.001
	3–5	1242	28	164	192	15.46		
	6–12	900	8	72	80	9.67		
	13–24	894	9	77	86	9.34		
	25–44	1641	34	120	154	9.02		
	45–64	1495	11	99	110	7.36		
	≥ 65	1083	9	103	112	10.43		
Total		13,083	204	1584	1788	13.67		

(112/1,083, 10.43%). The NoV detection rate significantly differed among the age groups ($P<0.001$) (Table 3).

Time distribution

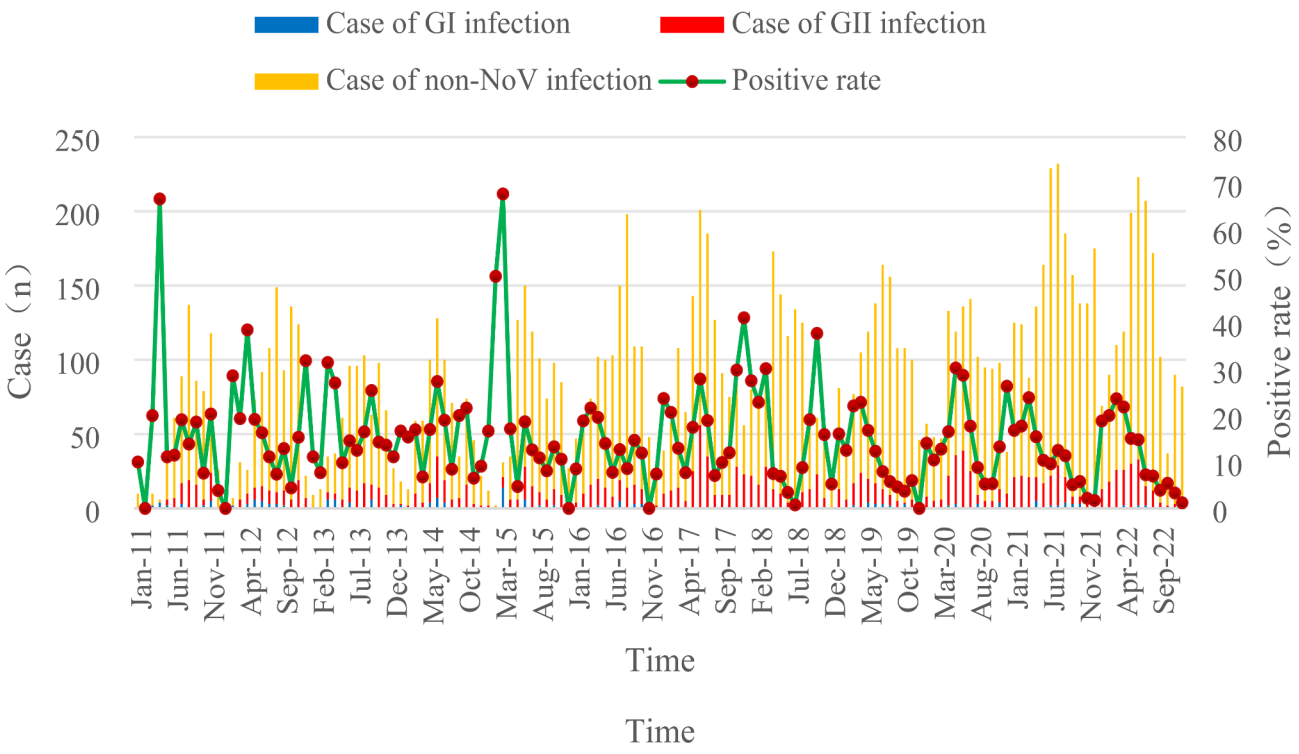
Overall, the peak incidences of NoV-induced acute diarrhea were concentrated in March and April. However, in 2014, 2017, and 2018, the incidence rates peaked in June, December, and October, respectively. June 2015, June 2017, and June 2020 also had high detection rates (Fig. 2). The NoV detection rate significantly differed among the seasons ($P<0.001$). GI detection was higher in March and April than in other months, whereas GII detection was higher from January to March than in other months. Generally, the NoV detection rate was high during winter (January and February) and spring (March and April), with March having the highest detection rate (Table 4).

Regional distributions

Overall, the NoV detection rate significantly differed among the regions of Ningxia Province ($P<0.001$). Guyuan had the highest detection rate (18.66%), and Yinchuan had the lowest (10.33%) (Table 5).

Prevalence and evolution of NoV genotypes

Genetic sequencing of the RdRp-capsid region of the NoV-positive samples resulted in 846 strains, including 68 GI strains and 778 GII strains. Moreover, 16 genotypes each were identified in the RdRp and VP1 regions, all predominantly as GII clusters (Fig. 3). The most common GI genotypes were GI.3[P3] (26.47%), GI.6[P11] (23.53%), and GI.3[P13] (20.59%), and the most common GII genotypes were GII.4[P16] (26.22%), GII.3[P12] (21.34%), and GII.4[P31] (18.25%). In addition, GII.6[P7], GII.4[P4], GII.8[P8], GII.7[P7], and GII.13[P16] were detected only in individual years. Two rare cases of the GII.22[P22] and GII.15[P15] genotypes were also monitored.



Time

Fig. 2 Temporal distribution of acute diarrhea cases in Ningxia Hui Autonomous Region from 2011 to 2022. The positive rate represents the number of NoV-positive cases. G: Genome; NoV: Norovirus

Table 4 Seasonal distribution of norovirus-induced acute diarrhea in Ningxia Hui Autonomous Region, China, from 2011 to 2022

Season	Month	Cases	NoV infection Cases		Total		χ^2	*P*
			GI	GII	NoV infection Cases	Positive rate(%)		
Spring	March	763	21	150	171	22.41	45.200	< 0.001
	April	996	20	138	158	15.86		
	May	1403	24	178	202	14.40		
Summer	June	1673	31	247	278	16.62		
	July	1725	26	189	215	12.46		
	August	1626	24	130	154	9.47		
Autumn	September	1081	12	100	112	10.36		
	October	1107	18	105	123	11.11		
	November	1051	19	113	132	12.56		
Winter	December	567	2	51	53	9.35		
	January	551	6	91	97	17.60		
	February	540	1	92	93	17.22		
Total		13,083	204	1584	1788	13.67		

The genotype distributions varied over time (Fig. 4). GII.4 Den Haag[P4] and GII.4 New Orleans[P4] were dominant in 2011, replaced by GII.4 Sydney[P4] in 2012, which disappeared in 2015 after a brief epidemic. Furthermore, GII.4 Sydney[P31] was prevalent from 2012, increasing gradually after 2016, peaking in 2018, then decreasing abruptly after 2021; this genotype was not detected in 2022. GII.4 Sydney[P16] was occasionally detected from 2017 to 2019 but significantly increased in 2020, becoming the dominant type in 2021 and peaking in 2022. GII.3[P12] was detected in all years except 2012, with high prevalence in individual years. GII.17[P17] became prevalent in 2013 and peaked in 2015, after which its prevalence weakened but remained detectable until 2022. The genotypes in the GI group were all detected in individual years; GI.3[P3] had a high detection rate in 2015, and GI.6[P11] was the predominant strain in the GI group, with small epidemics in 2015,

Table 5 Regional distributions of norovirus-induced acute diarrhea in Ningxia Hui Autonomous Region, China, from 2011 to 2022

Region	Cases	NoV infection Cases	Total			χ^2	*P*
		GI	GII	NoV infection Cases	Positive rate(%)		
Yinchuan	2566	27	238	265	10.33	66.723	< 0.001
Shizuishan	2855	35	339	374	13.10		
Wuzhong	2451	67	298	365	14.89		
Guyuan	1790	20	314	334	18.66		
Zhongwei	3421	55	395	450	13.15		
Total	13,083	204	1584	1788	13.67		

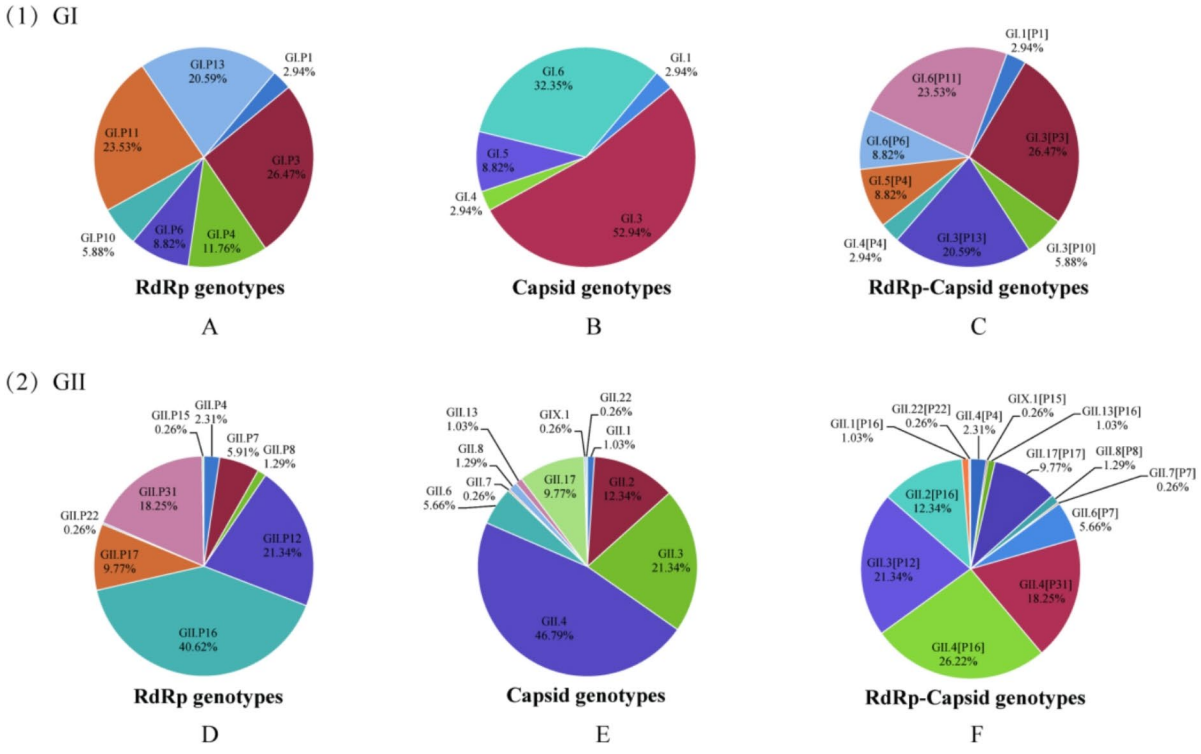


Fig. 3 Norovirus genotype distributions in Ningxia Hui Autonomous Region from 2011 to 2022. (1) GI: (**A**) RdRp, (**B**) Capsid, and (**C**) RdRp-Capsid genotypes. (2) GII: (**D**) RdRp, (**E**) Capsid, and (**F**) RdRp-Capsid genotypes. G: Genome; RdRp: Polymerase

2016, 2021, and 2022. Other GI strains were occasionally detected during this period.

The top four NoV types were GII.4, GII.3, GII.2, and GII.17. GII.4, GII.3, and GII.2 were the most prevalent in children aged 0–5 years, whereas GII.17 was most prevalent in adults aged 18–64 years (Fig. 5).

Phylogenetic analyses

Phylogenetic analyses of partial gene sequences in the RdRp and VP1 regions of GI and GII NoVs showed that all prevalent genotypes clustered with their prototype and representative strains. Furthermore, the distribution of homotypic strains detected in different years was relatively concentrated. Based on the partial RdRp region, both GI.P3 and GI.P4 strains in GI group formed two major evolutionary branches (Fig. 6A). In GII group, With the exception of GII.P15 and GII.P22 strains, all the other strains formed multiple evolutionary branches during the evolutionary process, forming relatively independent evolutionary branches although in general there was a high homology with their respective reference strains (Fig. 6C).

Based on the partial VP1 region, GI.3 was divided into four lineages, with three major evolutionary branches based on the time periods: 2012, 2015, and 2019–2020. GI.6 strains are divided into two major evolutionary branches (2015–2016 and 2021–2022) (Fig. 6B). In addition, during the monitoring period, the distribution of detected GII.3 strains was more concentrated, with a high homology between strains. GII.6 strains were

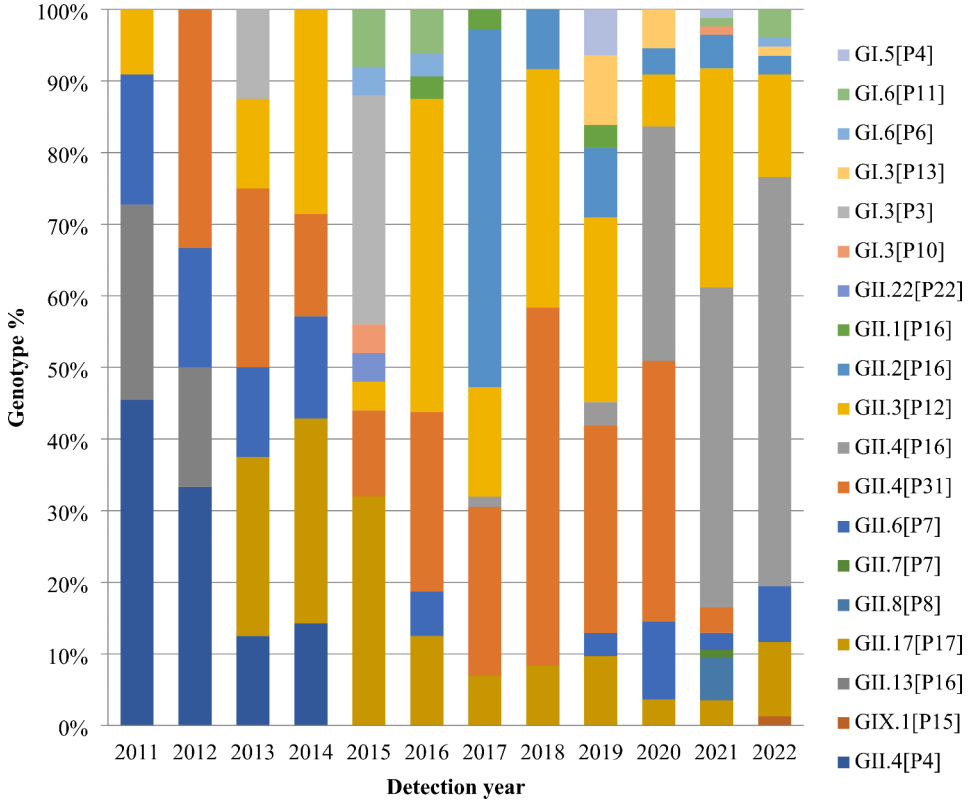


Fig. 4 Norovirus genotype distributions in Ningxia Hui Autonomous Region per year from 2011 to 2022. G: Genome

divided into major evolutionary branches, where strains located in the same evolutionary branch all have a large time span. GII.4 strains are also divided into two major evolutionary branches in the evolutionary tree (2011–2020, 2017–2022), and most of the strains of this type are located in the evolutionary sub-branch of 2017–2022, which has a small time span. Additionally, there are individual strains that form a separate evolutionary branch (Fig. 6D).

Discussion

In recent years, NoV clusters and outbreaks have increased, becoming a serious public health issue owing to the increased disease burden. However, the prevalence of sporadic cases and subsequent disease burden might be neglected. One study reported a 16% increase in the overall prevalence of sporadic NoV cases globally in 2019 compared to that in 2014 [15]. In China, the sporadic NoV detection rate was 16.68% [16], but the prevalence varied across regions, with the highest rates in Zhejiang Province in eastern China (22.05%) and Guangdong Province in southern China (26.08%) [16]. In this study, the overall NoV detection rate was 13.67%, of which 1.56% and 12.11% were in groups GI and GII, respectively. Thus, the prevalence of NoV in Ningxia Hui Autonomous Region is at a medium level. Ningxia Hui Autonomous Region is in western China and has a relatively backward economy, emphasizing the relationships between NoV prevalence and economic development and population density.

This study included all ages. Children aged 0–2 years had the highest overall positive detection rate (18.09%, 1,054/5,828), which significantly decreased after age 2 years. The overall detection rate in the 0–5-year-old group was 17.62% (1,246/7,070), similar to the global prevalence of NoV in patients under age 5 years (17.7%) [17]. The 45–64-year-old group had the lowest infection rate overall.

Over the 12 years of surveillance, NoV infections peaked in 2017, followed by a small peak in 2020, generally showing a pattern of peak incidence every two years. The prevalence of NoV in Ningxia Hui Autonomous Region was the lowest in all years in 2021, when Ningxia was under the most stringent preventive and control measures for the coronavirus disease 2019 pandemic.

This study also explored mixed infections, finding that GI NoVs frequently co-infected with GII, whereas co-infections with other gastroenteritis viruses were rare. Co-infection with GI and GII groups is more often reported in foodborne transmission. Mixed infections with rotavirus and astrovirus were the most common in the GII group, contrary to other regional studies that reported more GII NoV co-infections with rotavirus and adenovirus [18]; S.-X. Zhang, Yang, et al., [19]; S.-X.

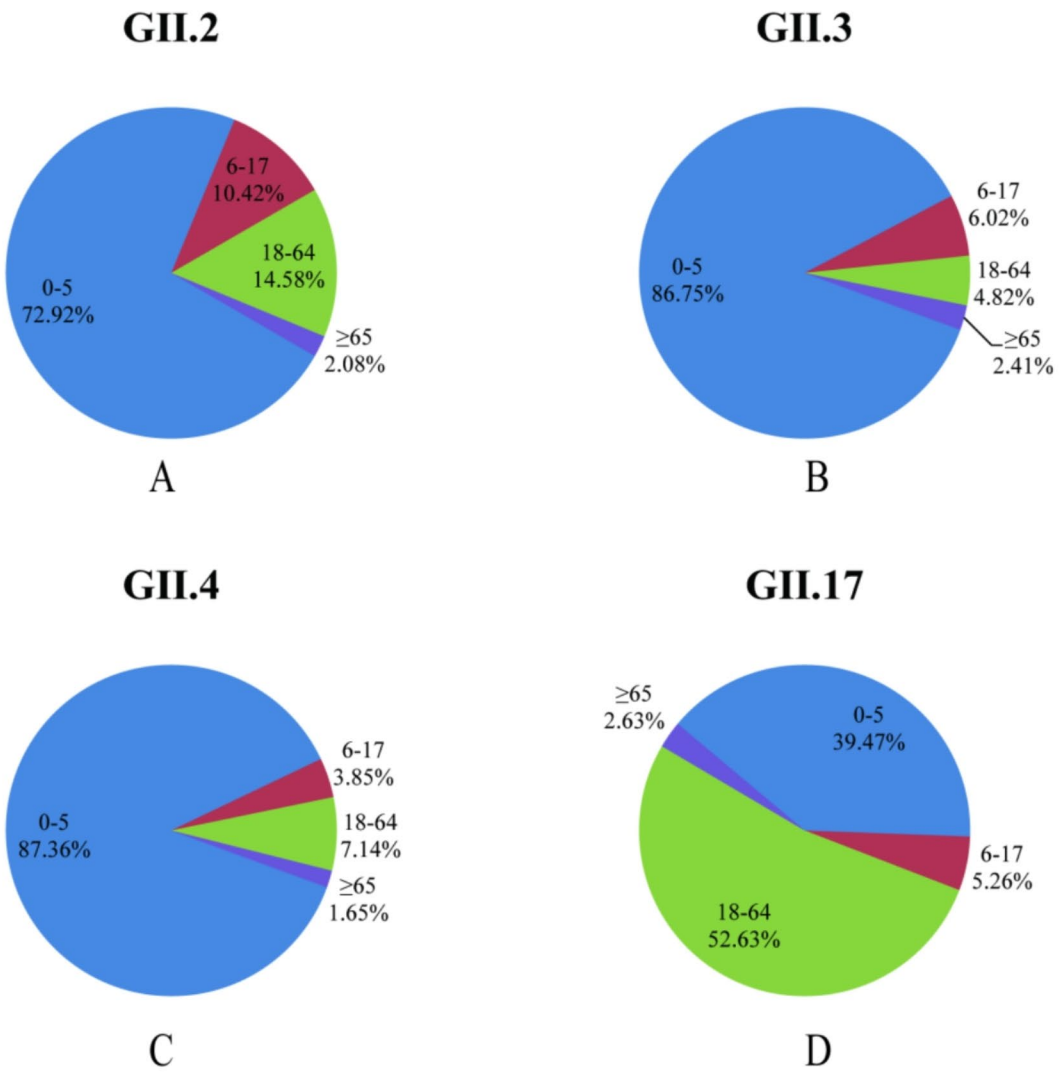


Fig. 5 Age distributions of the top four genotypes in the capsid region of noroviruses identified in Ningxia Hui Autonomous Region from 2011 to 2022: (**A**) GII.2, (**B**) GII.3, (**C**) GII.4, and (**D**) GII.17. G: Genome

Zhang, Zhou, et al., [20]. Mixed rotavirus and NoV infections have also been reported in India, Bangladesh, and Pakistan [18]; Chaurasia, Srivastava, & Kumar Singh [21], Dey et al., [22, 23]. As the detection techniques have improved, more viral co-infections have been identified in the gut, leading to a growing understanding of the prevalence of co-infections of gastroenteritis virus. However, the mechanisms underlying viral co-infections have not been carefully investigated. Recent research suggests that viral-viral interactions are synergistically pathogenic and may play an important role in developing host-viral group homeostatic relationships and the immune system (Makimaa, Ingle, & Baldridge [24]), . This area of research should be investigated further.

NoV-induced acute diarrhea occurs mainly in winter. Therefore, it was first known as winter vomiting [25], and studies have confirmed that NoVs are usually detected during the colder seasons (Kreidieh, Charide, Dbaibo, & Melhem [26], Li et al., [27]. In this study, NoV infections were detected throughout the year, but the seasons of prevalence differed between the GI and GII groups. The GII group was predominant in winter and spring, with a distinct seasonal pattern, whereas the GI group was predominant in the alternating months of spring, summer, and autumn. In addition, although the months with a high incidence of NoV-induced acute diarrhea varied slightly each year, in general, the high-incidence seasons in this region were winter and spring, consistent with incidence rates in other regions (Ahmed, Lopman, & Levy [28], Qin et al., [29]. However, some studies have reported NoV epidemic seasons in some regions of China during the autumn and winter [30, 31], perhaps

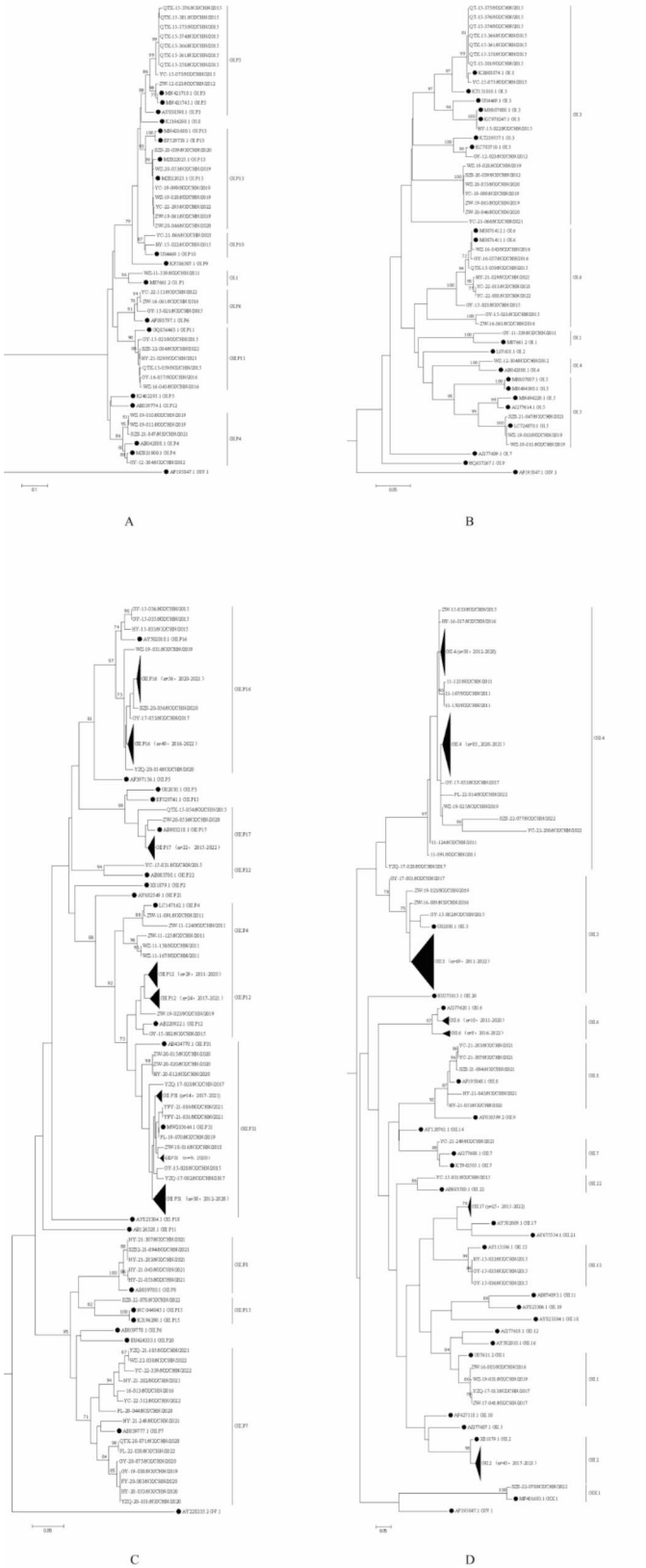


Fig. 6 Neighbor-Joining phylogenetic trees of partial norovirus strains identified in Ningxia Hui Autonomous Region, China. (**A**, **B**) Partial RdRp (265 bp) and VP1 (291 bp) region of GI. (**C**, **D**) Partial RdRp (262 bp) and VP1 (300 bp) region of GII. Symbols indicate reference strains for the respective genotypes. Bootstrap support values > 70 (1,000 replicates) are shown next to the branches. G: Genome; RdRp: Polymerase; VP1: Capsid

owing to local variations in rainfall, relative humidity, and temperature.

The GI and GII distributions also differed throughout the region. Wuzhong and Zhongwei had a high prevalence of GI infection, whereas Guyuan had a high prevalence of GII infection. However, Yinchuan, the most densely populated city in Ningxia Hui Autonomous Region, had the lowest positivity rate for both groups. Ningxia Guyuan, located south of Ningxia, is the intersection of the provincial capitals Xi' an, Lanzhou, and Yinchuan. In addition, the climate is cooler, and there is more rain, which may explain the high positivity rate in Guyuan.

The genotypes of NoV worldwide is diverse. Over the 12-year study period in Ningxia, 32 genotypes were detected, including 16 RdRp and 16 VP1 genotypes. Moreover, two to four genotypes were detected annually. In GI-positive samples, GI.3, GI.6, and GI.5 were the common genotypes, with GI.3 accounting for 52%. Globally, these genotypes are common in children [17] but are rare in adults. This study also found the same genotypes in several adolescents and adults.

The genetic diversity was greater in the GII than in the GI group. Among the GII group, GII.4 was the dominant genotype, followed by GII.3, GII.2, and GII.17, which peaked in 2015 and has been prevalent in Ningxia Hui Autonomous Region since its emergence in 2013. Moreover, in adults, GII.17 was detected more frequently than other types, such as GII.4 and GII.3. Other studies have also reported significantly higher GII.17 detection rates in adults than in children [32, 33], which could be related to the time of the GII.4 epidemic, where adults had immunity against the GII.4 strain but less against the novel GII.17 strain. Moreover, children have low immunity, making them more susceptible to GII.4, which is more contagious than GII.17 [34].

The most dominant genotype in the RdRp region was GII.P16, followed by GII.P12 and GII.P31. GII.P16 appeared in 2015, accounting for only a small percentage of cases in Ningxia Hui Autonomous Region, but suddenly increased in 2017. Notably, the NoV prevalence peaked in Ningxia Hui Autonomous Region in 2016, which might be related to the prevalence of GII.P16. GII.P16 and GII.P12 are the viruses with the highest evolutionary rate among the 25 RdRp genotypes, and viruses recombined with the new GII.P16 may enhance viral adaptability [35].

GII.4[P16], GII.4[P31], GII.2[P16], GII.3[P12], and GII.6[P7] are the five most common dual fractional genotypes worldwide [9]. The most predominant genotype in Ningxia Hui Autonomous Region was GII.4[P16], followed by GII.3[P12], GII.4[P31], and GII.2[P16], largely consistent with global and Chinese prevalence trends. However, in Ningxia Hui Autonomous Region, GII.3[P12] was the dominant genotype after GII.4[P16], slightly differing from the dominant genotypes in other regions [9]; Zhou, Wang, von Seidlein, & Wang [36]), .

This study also foundthat the predominant genotypes changed over the 12-year study period. For instance, two GII.4 variants were identified in 2011, GII.4 Den Haag and GII.4 New Orleans, both of which combined with GII.P4 and were replaced by the GII.4 Sydney[P31] or GII.4 Sydney[P16] in 2012, and replaced the GII.4[P4] strain as the new predominantly prevalent GII.4 strain. Moreover, GII.4 Sydney[P31] was detected during the study period, surpassing the other genotypes as the new dominant strain in 2018, which lasted until 2020; it decreased rapidly after 2021 and was not detected in 2022. GII.4 Sydney[P16] emerged in 2017 and increased gradually in 2020, becoming the predominant strain in 2021 and 2022.

In addition, GII.3[P12] appeared in 2011 with stable and sustainable transmission and was never replaced, nor did it disappear. Although it was not the most prevalent genotype each year, the number of detections during the monitoring period was second only to that of GII.4[P16], the second most prevalent genotype in Ningxia Hui Autonomous Region. In 2017, GII.2[P16] was the predominant type, decreasing yet persisting from 2018 until 2020, when the number of GII.2[P16] cases significantly decreased in the domestic reports. In this study, the dominant genotype in Ningxia Hui Autonomous Region changed over time, from GII.4 Sydney[P31] to GII.4 Sydney[P16]. In other places, the dominant type changed from GII.4 Sydney[P31] to GII.2[P16]. These results suggest that new genotypes are the most likely to cause widespread epidemics. Furthermore, new genotypes emerged every few years, replacing the previously dominant genotype, primarily due to recombination. Therefore, this study also monitored several recombinant genotypes in addition to the major genotypes, including GII.6[P7] and GII.13[P16]. Several rare strains were also detected, such as GII.22[P22] and GIX.1[P15].

Based on the evolutionary analyses of RdRp and VP1 regions, it was found that many common strains in this region had high affinities with the representative strains of the same type, but formed relatively independent evolutionary branches, with certain temporal distributions, which suggested that there was a trend of small-scale epidemics in Ningxia during the detection period. In addition, GII.4 strains have a more complex composition in the evolutionary tree, and most of the strains are in different small branches under the same main branch, which indicated that they will be more easy and frequently to mutation, and we should pay attention to the mutation situation of this group of strains.

Limitations of this study: This study was based on sequencing of the norovirus genome segment (the

overlap of the polymerase region (RdRp) and capsid region (VP1) sequences). Although the genetic information of norovirus genome is limited so that they do not support further in-depth study, the current data still show that there are more genotypes and rich genetic diversity of norovirus in this region. Therefore, we will increase the monitoring and collection of norovirus in Ningxia in the future, and comprehensively understand the genetic evolution characteristics of norovirus in Ningxia through whole genome sequencing and other means. To provide scientific basis for vaccine research and development and disease prevention and control.

In conclusion, surveillance data from 2011 to 2022 highlights the diversity of NoVs prevalent in Ningxia Hui Autonomous Region, China. The dominant genotypes were consistent domestically and internationally but were constantly replaced over time, which is a challenge for vaccine development37. Therefore, strengthening molecular surveillance is particularly important for future vaccine design.

Author contributions

Investigation: Jiangtao Ma, Qian Chen, Fang Yuan, Min Cao, Cong Yang, Jiangwei Gao, Ran Xian and Lei Gao. Resources: Jiangtao Ma, Jiangwei Gao and Cong Yang. Writing–review and editing: Jiangtao Ma and Ming Tan. Project administration: Jiangtao Ma and Wenhe Kuai. Writing–original draft: Jiangtao Ma. Funding acquisition: Jiangtao Ma, Jiangwei Gao and Cong Yang. Supervision: Jiangtao Ma, Ming Tan, and Wenhe Kuai. Conceptualization: Qian Chen. Formal analysis: Qian Chen, Fang Yuan, Min Cao, Cong Yang, Jiangwei Gao, Ran Xian and Lei Gao. Visualization: Qian Chen and Ran Xian. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Viral diarrhea caused by NoV in China has been a public surveillance program so that we don't need to get an ethical approval.

Competing interests

The authors declare no competing interests.

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