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Epidemiology and genetic diversity of group A rotavirus in acute diarrhea patients in pre-vaccination era in Himachal Pradesh, India

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ABSTRACT

Acute gastroenteritis due to Group A rotaviruses remains the leading cause of mortality and morbidity in children in developing countries. India introduced its indigenous rotavirus vaccine Rotavac® in 2016 and Himachal Pradesh (HP) the first state to launch it. The present study aimed to evaluate rotavirus strain diversity associated with AGE prior to vaccine introduction in HP. A total of 331 fecal specimens collected from diarrheic children hospitalized at RPGMC Tanda, HP between July-2014 and June-2016 were screened for RVA by EIA. Rotavirus RNA was extracted by TRIZOL method and analyzed by RNA-PAGE. G/P typing was performed using semi-nested multiplex reverse transcriptase PCR. Rotavirus was detected in 45% (n = 149/331) of diarrheic children, with highest rate observed in the 6–11 months age group (47%). Vomiting was found more frequently associated with RV-infection. Among G-types, G12 was found most prevalent (33.1%) followed by G1 (28.4%), G9 (12.2%), G2 (9.5%), G3 (3.4%) and G10 (2.7%). G4 (0.7%) strains were rarely detected. Among P-types, P[6] was the most prevalent (40.5%) followed by P[8] (29.1%) and P[4] (14.2%). Of note, genotypes G3 and P[11] were detected for the first time in HP. Among G/P combinations, G12P[6] was most prevalent (30.4%) followed by G1P[8] (20.3%), G2P[4] (4.7%), G1P[6] (3.4%) and G3P[8] (2.7%). Interestingly, our study observed high percentage of unusual strains (14.2%) namely G9P[4], G2P[6], G2P[8], G12P[4] and G1P[11]. The regionally common strains G3P[6], G4P[6], G9P[6], G9P[8], G10P[6], G10P[8] and G12P[8] strains were very rarely detected. Of interest, RNA migration pattern of G1P[8] was DS-1 like and genomic heterogeneity was observed within G12P[4] strains with both long and short electropherotypes. Our study highlights rich genetic diversity with emergence of rare rotavirus strains circulating in HP and provides baseline data prior to Rotavac® introduction that will help to gauge the impact of the Rotavac® vaccine in HP.

1. Introduction

Rotavirus remains the most severe cause of acute gastroenteritis (AGE) among infants and young children worldwide [1]. Every year in India, rotavirus (RV) accounts for 78,500 deaths, 872,000 hospitalizations, and 3.2 million outpatient cases in children <5 years of age [2]. On the basis of antigenic differences of VP6 proteins, rotaviruses are classified into eight groups, A to H [3]. Acute gastroenteritis in human is caused by group A, B and C rotaviruses, however, most of the disease burden particularly in children are primarily caused by group A rotavirus. Group A Rotavirus (RVA), member of family *Reoviridae,* consists of 11 segments of dsRNA (double-stranded RNA). The outer capsid proteins, VP7 (glycoprotein) and VP4 (protease sensitive protein) form the basis of G and P dual typing system, respectively. To date, RVA have been classified into 27G and 37P genotypes based on the differences in nucleotide sequences of VP7 and VP4 genes [4]. Globally, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] strains are the most common cause of human infection [5]. However, a number of other rotavirus strains specifically unusual, rare or novel strains have been generated from time to time by the mechanism that may include reassortment as a results of coinfection with human and animal strains [6–8].

Abbreviations: AGE, acute gastroenteritis; RV, rotavirus; RVA, rotavirus group A; RT-PCR, reverse transcriptase polymerase chain reaction; cDNA, complementary DNA; EIA, enzyme immunoassay; HP, Himachal Pradesh; UIP, universal immunization programme.

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India introduced its live attenuated indigenous rotavirus vaccine, Rotavac® (G9P[11]) into its Universal Immunization Programme (UIP) in 2016 and Himachal Pradesh (HP) is first state to launch it. There may be possibility of change in diversity of circulating rotavirus strains due to the vaccine selection pressure [9]; hence, it is very important to have the pre-vaccination data of the region. However, change in the diversity of rotavirus strains can occur without the use of any rotavirus vaccine "through natural selection" as observed by Kaplon et al. [10]. The present study aims to evaluate the prevalence and distribution of rotavirus strains particularly unusual types circulating in HP, a mountainous state of Northern India. This pre-vaccine data will provide a baseline to compare with post-vaccine data and will help to gauge the impact of Rotavac® vaccination in HP.

2. Materials and methods

2.1. Study samples

A total of 331 fecal specimens were collected with the written consent of parents/guardians from children (<5 years) hospitalized for AGE at Rajendra Prasad Govt. Medical College (RPGMC) Tanda, HP from July-2014 through June-2016. Fecal specimens from diarrheic children were collected within 48 h of hospital admission. Monitoring of rotavirus cases was performed from July-2014 to June-2015 (referred as 2015; n = 211) and July-2015 to June-2016 (hereafter, 2016; n = 120). The presence of three or more episodes of unformed stool in any 24 h period was considered as acute diarrhea. Each patient's clinical data was recorded to define disease severity using Vesikari Scoring system with scores 0–5 considered mild, 6–10 moderate, 11–15 severe and 16–20 very severe [11]. The stool samples were stored at −70 °C for further use.

2.2. Rotavirus detection

A 10% supernatant of fecal specimens was screened for RVA antigen by monoclonal antibody based enzyme immunoassay (EIA) kit (Premier Rotaclone, Meridian Bioscience Inc., Cincinnati, OH, USA) as per manufacturer's protocol [12].

2.3. Viral RNA extraction

Rotavirus dsRNA was extracted in duplicates from 10% fecal suspensions of rotavirus positive specimens using Trizol method (Invitrogen Corp, Carlsbad, CA) according to manufacturer's instructions [7]. One aliquot of extracted RNA was used for further preparation of complementary DNA (cDNA).

2.4. Polyacrylamide gel electrophoresis (PAGE)

To determine the electropherotype of rotavirus strains, the other aliquot of extracted RNA was electrophoresed on 10% polyacrylamide gel according to the method described previously [12].

2.5. Genotyping

Complementary DNA was synthesized from extracted viral RNA through reverse transcription using random hexamer primers (Pd (N)6 hexamers, Invitrogen Life Technologies) and 200 units of Moloney murine leukemia virus reverse transcriptase (Invitro Life Technologies) according to procedure described in previous studies [13,14]. This cDNA was used as template for G/P genotyping PCR.

The consensus primers VP7-F/VP7-R and Con2/Con3 were used to amplify VP7 and VP4 genes, respectively in first round PCR [15,16]. Further, G/P typing was performed by semi-nested multiplex PCR using genotype specific primers described previously [13]. The PCR products were electrophoresed on 2% agarose gel containing ethidium bromide and visualized under ultraviolet gel documentation system. Two percent of genotyped samples were confirmed by sequencing**.** The samples that could not be genotyped with the G and P specific primers were noted as non-typeables.

2.6. Statistical analysis

Chi-square and Fischer's exact tests were used for statistical analysis. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Detection of rotavirus antigen

Overall, 149 (45%) cases were found positive for RVA among 331 fecal specimens tested by EIA, with the high proportion in year 2015 (48.3%) followed by 2016 (39.2%) (Table 1). Year wise analysis showed no statistical difference in rotavirus positivity between the study years. Rotavirus was detected throughout the year with high percentages of infection in autumn (September-November) and winter (December-February) seasons.

3.2. Demographic characteristics and clinical data

Distribution of diarrheic children according to age, sex and clinical characteristics is shown in Table 2. The percentage of rotavirus positive cases was highest in age group 6–11 months followed by 12–23 months ($P = 0.002$). Children <2 years of age presented 86.6% of the total disease burden. The mean age (±standard deviation) of rotavirus-infected children was 13.32 ± 9.55 months, which was significantly lower ($P = 0.014$) as compare to rotavirus-uninfected children (16.48 ± 12.74 months). Children of age group 24–59 months were significantly less infected with rotavirus ($P = 0.017$). There was no significant difference in the detection rate of rotavirus by sex (46.7% among boys vs. 41.9% among girls), however, the number of boys admitted was more compared to girls (214/331 [64.6%] vs. 117/331 [35.3%]). Diarrhea was found to be significantly more severe in rotavirus-infected children than rotavirus-uninfected children (mean severity scores, 12.82 vs. 12.03; $P = 0.001$). Frequency of vomiting episodes ($\geq$5/day) were significantly more in RV infected children than RV uninfected children (Table 2).

3.3. Genotyping

Of the 149 rotavirus positive samples, G/P genotyping could be performed for 148 (99.3%) samples with one sample was not genotyped because of insufficient quantity (Table 3). G-genotypes were identified in 137/148 (92.6%) samples whereas P-genotypes

Table 1
Rotavirus positivity in children with acute gastroenteritis in Tanda (Himachal Pradesh), Northern India (July 2014 – June 2016).

Study Year	No. of samples tested	No. of samples positive (%)
2015[a]	211	102 (48.3)
2016[b]	120	47 (39.2)
Total	331	149 (45.0)

[a] 2015: July 2014 – June 2015.
[b] 2016: July 2015 – June 2016.

Table 2
Characteristics of rotavirus-infected and -uninfected children (<5 years) hospitalized with acute gastroenteritis.

Variable	Rotavirus infected children (n = 149)	Rotavirus uninfected children (n = 182)	*P* value
Sex			
Female	49	68	
Male	100	114	0.420
Age, mean months ± SD	13.32 ± 9.55	16.48 ± 12.74	**0.014**
Age (months)			
0–5 (n = 41)	15 (10.1)	26 (14.3)	0.314
6–11 (n = 127)	70 (47.0)	57 (31.3)	**0.004**
12–23 (n = 99)	44 (29.5)	55 (30.2)	0.904
24–59 (n = 64)	20 (13.4)	44 (24.2)	**0.017**
Vomiting (for ≥ 3 days)	34 (22.8)	27 (14.8)	0.065
Episodes/day (≥ 5)	58 (38.9)	48 (26.4)	**0.017**
Vesikari score, mean ± SD	12.82 ± 2.08	12.03 ± 2.39	**0.001**
Disease severity by Vesikari score			
Mild	0 (0)	0 (0)	
Moderate	18 (12.1)	44 (24.2)	**0.006**
Severe	115 (77.2)	123 (67.6)	0.065
Very severe	16 (10.7)	15 (8.2)	0.454

Data are no. (%) of children, otherwise indicated SD, standard deviation.
The values in boldface indicate the statistically significant.

were identified in 130/148 (87.8%) fecal specimens. Both G and P types were determined in 129 of 148 rotavirus positive sample. Ten samples (6.7%) were non-typeable for both the types. Among G-types, G12 was the most widely circulating genotype (33.1%) in HP followed by G1 (28.4%), G9 (12.2%), G2 (9.5%), G3 (3.4%) and G10 (2.7%), while G4 (0.7%) was rarely detected. G-types as proportions of total number of G-mix were, G1/G12 (1.4%), G1/G9 (0.7%) and G1/G3 (0.7%). Among P-types, P[6] accounted for highest prevalence (40.5%) followed by P[8] (29.1%) and P[4] (14.2%). P[11] (0.7%) was rarely detected in HP. Coinfection of various P-types (P[6]/P[8], P[6]/P[4] and P[4]/P[8]) was seen in 3.4% of genotyped samples (Table 3).

In G/P combinations, we detected 17 different rotavirus strains at varying frequencies. Among the globally common G/P combinations, G12P[6] emerged as the most predominant rotavirus strain (30.4%) followed by G1P[8] (20.3%), G2P[4] (4.7%), G1P[6] (3.4%) and G3P[8] (2.7%). The regionally common rotavirus strains, G3P[6], G4P[6], G9P[6], G9P[8], G10P[6], G10P[8] and G12P[8] were very rarely detected and in total constituted 6.3% of the total strains genotyped. Interestingly, our study demonstrated high percentage of unusual strains (14.2%) namely G9P[4] (8.1%), G2P[6] (2%), G2P[8] (2%), G12P[4] (1.4%) and G1P[11] (0.7%). Of note, non-typeable rotavirus strains for both types were detected at low frequency (6.7%) (Table 3).

3.4. Temporal distribution of rotavirus strains

The yearly distribution of rotavirus G and P genotypes throughout the study period is shown in Fig. 1A and B, respectively. G12 was the most common strain detected in 2015 (43.1%) whilst in 2016, the common strain detected was G1 (30.4%). G1 and G9 remained fairly constant throughout study periods. The frequency of G2 was found to have increased in 2016 (13%) as compare to 2015 (7.8%). G4 and G3 were detected only in 2015 and 2016, respectively. Of interest, the percentage of mixed infections of G-types doubled in year 2016 (4.3%) from 2015 (2%). The proportion of G non-typeable was found to be increased in 2016 (13%) from 2015 (4.9%) (Fig. 1A). The frequency of P[6] was observed to have decreased from year 2015 (50%) to 2016 (19.6%) with the increase in genotype P[8] (20.6% in 2015 and 47.8% in 2016). P[4] was detected at higher frequency in 2016 (19.6%) as compare to 2015 (11.8%). Importantly, rare genotype P[11] was detected for the first time in HP in year 2015. The frequency of P non-typeables increased from year 2015 (11.8%) to year 2016 (13%) (Fig. 1B).

Temporal distribution of rotavirus common (Fig. 2A) and rare (Fig. 2B) strains showed G12P[6] rotavirus prevalence in 2015 (40.2%) while G1P[8] were predominant (30.4%) in 2016. Rotavirus strains G1P[6], G4P[6], G9P[6] and G12P[8] were completely absent in the region in 2016. Interestingly during 2015–2016 both G2P[4] and G9P[8] rotavirus showed an increase from 3.9% to 6.5% and from 1% to 2.2% respectively. G3P[6], G3P[8], G10P[6] and G10P[8] strains were detected only in 2016 (Fig. 2A). Rare strain like G9P[4] was detected at nearly similar frequency during both the year 2015 (7.8%) and 2016 (8.7%). Of note, G2P[6] and G12P[4] were found to have increased from 2015 to 2016. G1P[11] and G2P[8] roravirus strains were detected only in 2015 (Fig. 2B).

3.5. Electropherotyping

Fig. 3 shows the RNA migration pattern of rotavirus globally common and rare strains detected in HP. Globally common strains, G1P[8] and G2P[4] were of short (S) electropherotype, while G3P[8] and G12P[6] presented long (L) electropherotype. Unusual strains, G9P[4] exhibited short electropherotype while G2P[8] had long electropherotype. Unusual strains G12P[4] showed both L and S electropherotypes.

4. Discussion

The World Health Organization proposes the use of rotavirus vaccines to protect children against severe disease caused by rotavirus globally [17]. Himachal Pradesh has introduced rotavirus vaccine Rotavac® and therefore, it would be necessary to monitor the circulating rotavirus strains to assess the impact of vaccine

Table 3
Overall distribution of G & P type of rotavirus strains circulating in Tanda, Himachal Pradesh during July 2014 to June 2016.

Rotavirus type	No. (%) of strains[e]									
	G1	G2	G3	G4	G9	G10	G12	Gmix[a]	Gnt[b]	Total
P[4]	0	*7 (4.7)*	0	0	**12 (8.1)**	0	**2 (1.4)**	0	0	21 (14.2)
P[6]	*5 (3.4)*	**3 (2.0)**	*1 (0.7)*	*1 (0.7)*	*1 (0.7)*	*1 (0.7)*	*45 (30.4)*	2 (1.4)	1 (0.7)	60 (40.5)
P[8]	*30 (20.3)*	**3 (2.0)**	*4 (2.7)*	0	*2 (1.4)*	*2 (1.4)*	*1 (0.7)*	1 (0.7)	0	43 (29.1)
P[11]	**1 (0.7)**	0	0	0	0	0	0	0	0	1 (0.7)
P[mix][c]	2 (1.4)	1 (0.7)	0	0	0	0	1 (0.7)	1 (0.7)	0	5 (3.4)
P[nt][d]	4 (2.7)	0	0	0	3 (2.0)	1 (0.7)	0	0	10 (6.7)	18 (12.2)
Total	42 (28.4)	14 (9.5)	5 (3.4)	1 (0.7)	18 (12.2)	4 (2.7)	49 (33.1)	4 (2.7)	11 (7.4)	148 (1 0 0)

[a] Gmix, G1/G12, G1/G3, G1/G9.
[b] Gnt, nontypeable for the G genotypes.
[c] Pmix, P[6]/P[8], P[6]/P[4], P[4]/P[8].
[d] P[nt], nontypeable for the P genotypes.
[e] Data shown in boldface type represent unusual G/P combinations and italic type represent common strains.

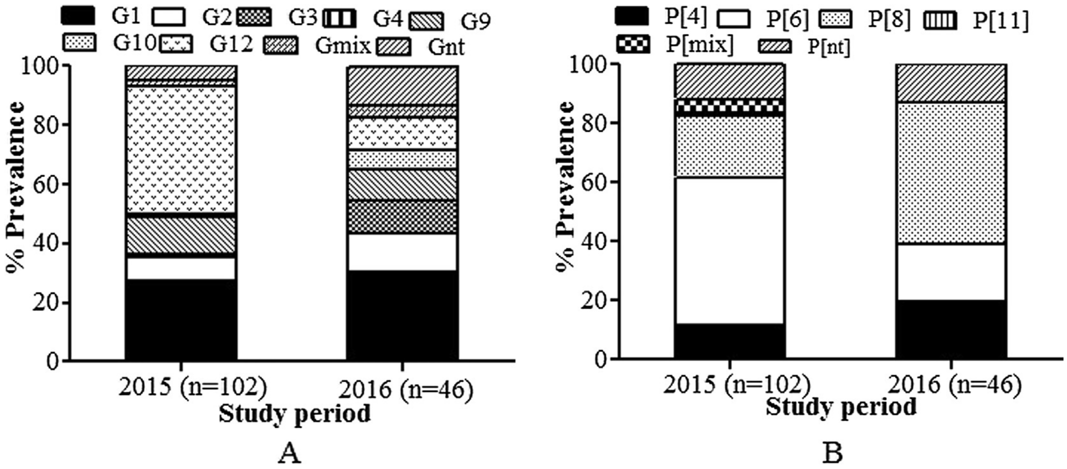

Fig. 1. (A) Yearly prevalence of G genotypes (G1, G2, G3, G4, G9, G10, G12, mixed [Gmix], and nontypeable [Gnt]) among children hospitalized with acute diarrhea in Himachal Pradesh. (B) Yearly prevalence of P genotypes (P[4], P[6], P[8], P[11], mixed [Pmix] and nontypeable [Pnt] among children hospitalized with acute diarrhea in Himachal Pradesh.

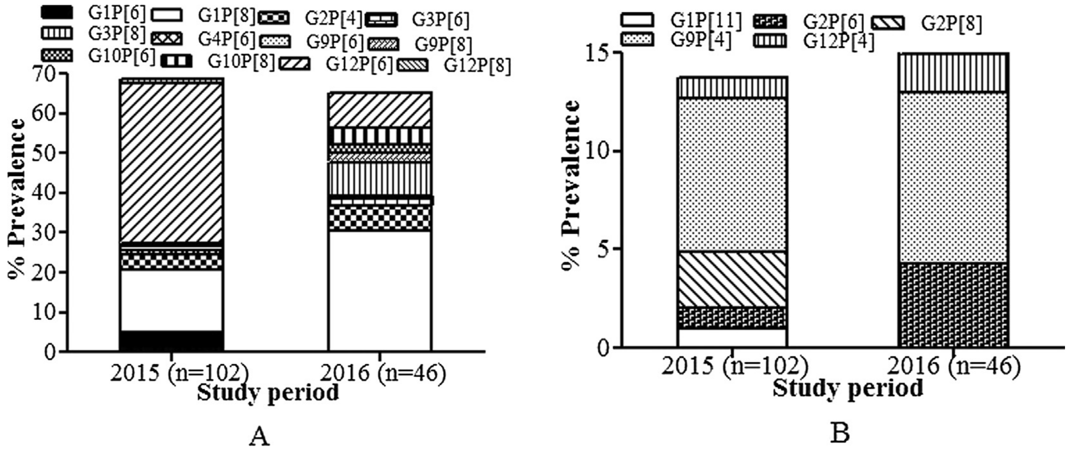

Fig. 2. Temporal distribution and prevalence of (A) common rotavirus strains and (B) rare/unusual rotavirus strains circulating in Himachal Pradesh during two years (July-2014 through June-2016).

on rotavirus diarrhea. There are only few reports on the epidemiology and strain diversity of rotavirus from HP with two multicenter studies using very few samples and one recent report by Jain et al. [18–20]. This study provides data on the prevalence of rotavirus in diarrheic children with their clinical attributes and strain diversity prior to vaccine introduction. We also studied the temporal distribution of common and unusual rotavirus strains circulating in HP.

Our study demonstrated a huge burden of rotavirus disease among Himachali children. Similar high incidences of rotavirus infection in diarrheic children have also been reported from other parts of the country [6,20–22]. A recent study of extended Indian Rotavirus Surveillance Network also reported that Tanda was the region to show highest rotavirus positivity during 2012–2014 [23]. The reason for highest rotavirus positivity in HP could be the cool, semi-humid and sub-tropical climate. The other reason could be the low level of socio-economic population. The highest burden of rotavirus disease was in children <2 years of age, a finding that is consistent with other studies [13,24,25]. Vomiting was frequently associated with RV-infection agrees with other report [26]. Our data showed the presence of severe form of diarrhea in both rotavirus positive and non positive cases. Several other studies including our own have reported severe form of diarrhea caused by other pathogens such as non group A rotavirus (rotavirus group B and C), bacteria and parasites that are not tested in the present study [13,27,28].The relatively low infection rate of rotavirus among older children (>2 years) could be partly due to immunity acquired through previous exposure(s). Velazquez et al. also reported that children get complete protection against moderate to severe form of diarrhea after two repeated rotavirus infections irrespective of symptomatic or asymptomatic infections [29].

Our study detected the most of the globally common G- genotypes namely G1, G2, G3, G4, G9 and G12. G12 was for the first time detected in year 2003 in symptomatic cases in India [30]. Subsequently it was emerged in various parts of the country in symptomatic children as well as asymptomatic newborns [12,31–33]. In our study G12 was found to be the most dominant genotype associated with diarrhea in children. However previous studies from India including HP didn't see such high percentage of G12 RV [5–7,12,20,31,32]. Interestingly, our previous study observed high frequency of G12 for the first time in asymptomatic neonates from Delhi [33]. A study performed on rotavirus diversity in the Indian subcontinent showed the emergence of G9 and G12 rotavirus strains with simultaneous decline in G3 and G4 RV [7,34]. Similar results with rare detection of G3 and G4 genotypes were observed in our study in HP. Interestingly, our study detected G3 RV for the first time in HP, however this does not rule out the

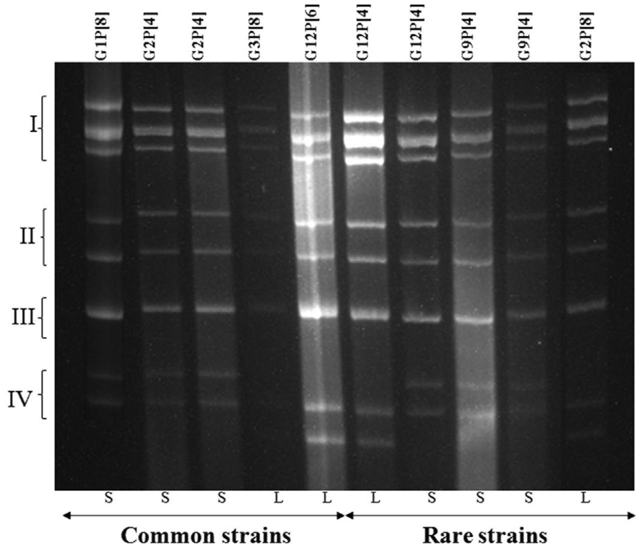

Fig. 3. Electropherotypes of rotavirus representative globally common and rare strains identified in Himachal Pradesh, having long (L) and short (S) electropherotypes. Long electropherotypes were shown in Lanes 4, 5, 6 and 10; and short profiles in lanes 1, 2, 3, 7, 8 and 9. I, II, III and IV indicate clusters of migrating gene segments of rotavirus RNA.

possibility that it might have missed out by other investigators from HP [18–20]. Similarly G4 was reported during 1993 from HP and now our study detected G4 after a gap of 22 years [19]. This shows the recurrence of rotavirus strains in HP. G9 was identified at similar frequency as in earlier studies from the country [12,22,35–37]. G10 was first detected in India in 1993 and subsequently it was detected in many other studies from India [38–40]. Of note, recent studies from India indicated its (G10 RV) origin from bovine and porcine strains through re-assortment [31,40]. Therefore, it is important to further characterize these G10 strains to identify their origin and evolution. High prevalence of mixed infections is a unique characteristic of rotavirus epidemiology in India [41]. We detected both mixed G-types and mixed P-types in our study. Mixed G-types appear to be increased from year 2015 to 2016; however this was not found significant. High prevalence of mixed infection warrants formulation of vaccines against a broad range of RV strains.

Worldwide, P[4] and P[8] are common P-genotypes while P[6] is commonly detected in Asia and Africa [7,34]. We observed P[6] the most predominant VP4 genotype followed by globally common P[8] and P[4] genotypes which is in agreement with other reports [18–20]; while our findings was in contrast to studies where P[8] was the most prevalent genotype [5,12,22]. In general P[4] RV was very rarely detected in HP, studies conducted during 1996–1998 and 2012–2015 did not even detect any P[4] [18–20]. Present study detected P[4] as the third most prevalent genotype in HP having combinations with G2, G9 and G12 . Such combinations have been reported from other parts of India [6,22,35]. Of note, P[11] strain was detected for the first time in HP. Frequency of G and P non-typeable strains appeared to be increased from year 2015 to 2016. The reason for higher number of non-typeables could be due to point mutation(s) at primer binding region of VP7/VP4 genes [8]. However in overall the frequency of G/P nontypeables seems lower as compare to other studies from North India, although this difference is not found significant [7,20].

Rotavirus strains vary with the time and geographical location. G12P[6] was the predominant strain in this study. This is the first time in India with such high RV-infection in children due to G12P[6]. However, G12P[6] showed temporal variation, being prevalent in 2015, while in 2016 G1P[8] was the predominant rotavirus strain. Such high incidence of G12P[6] was reported in newborns from Delhi and more recently from the south-east Asian region specifically in Nepal [1,33]. A recent study from HP by Jain et al. demonstrated a high incidence of diarrhea in children due to G1P[6] rotavirus [20]. However in our study, G1P[6] was found less prevalent and that too only during 2015, was completely absent in 2016. This marks a shift in the rotavirus strains over the time period in HP. Both, Rotarix and Rotavac$^{\circledR}$ are monovalent vaccines. Rotarix was found to be less effective against G2P[4] strains [42]. We detected G2P[4] at prevalence rate with increasing frequency from year 2015 to 2016 and it would be interesting to see the impact of monovalent vaccine Rotavac$^{\circledR}$ on such strains. The present study demonstrates the first detection of G3P[6], G3P[8], G4P[6], G10P[6], G10P[8] and G12P[8] strains in HP.

Our study observed high percentage of unusual rotavirus strains is in agreement with other studies from India [7,12,35]. The present study marked a sporadic detection of rare rotavirus strain G1P[11] in HP and this finding is in consistent with our earlier study from Delhi [7]. Interestingly, a similar study from Eastern India reported a high percentage of G1P[11] during 2013–2015 [14]. Among unusual rotavirus strains, G9P[4] was the predominant strain followed by G2P[6], G2P[8], G12P[4] and G1P[11]. The percent detection of G9P[4] in our study seems similar to other studies from India [7,21,35]. In recent years G9P[4] rotavirus slowly appears to prevail in the communities not only in India but also globally [6,43,44]. Frequent detection of G9 rotaviruses in HP as well as other parts of India justifies the G9 based vaccine, Rotavac$^{\circledR}$ in India [6,7,18,19,21,35,45]. The year 2015 exhibited rich diversity among circulating rotavirus strains as compare to 2016.

The genetic diversity and heterogeneity of rotavirus strains can be identified by the analysis of electropherotypes. The G1P[8] strains detected in the present study possessed short electropherotype which appears to be similar to the novel re-assortant DS-1 like G1P[8] strains from Japan, Thailand and Vietnam [46]. We observed G12 strains in combination with P[6] and P[4] genotypes and having long electropherotype like prototype L26. Recently Rahman et al. reported clustering of NSP2 and NSP5 genes between L26 and porcine rotavirus strains indicating their animal origin [47]. However, phylogenetic analysis of various genes of such atypical RV strains are required to demonstrate the origin of these strains. Our observation on rare G12P[4] strain with DS-1 like electropherotype is similar to that of Seheri et al. [48]. We agreed with their suggestion that these atypical strains might have emerged through re-assortment between Wa and DS-1 like strains [48].

A limitation of our study is that it includes only hospitalized cases and therefore the data is unlikely to be a true reflection of disease burden due to rotavirus or rotavirus strain types circulating in the community. Also, we have not analysed the association of rotavirus genotypes with clinical severity, however several earlier studies showed no association between clinical severity with rotavirus genotypes [49,50].

In summary, the present study demonstrated rich diversity of rotavirus strains with the detection of rare strains like G2P[8] and G12P[4] and continuous circulation of unusual RV like G9P[4] in HP. In addition, our study observed G12P[6] as the predominant cause of AGE in Himachali children. Our data suggests switching of RV strain prevalence from year to year. More importantly our data could be useful in documenting change in RV strain diversity due to possible vaccine selection pressure. In conclusion, future studies (post-vaccination) should be undertaken after 3–4 years to compare between the pre-vaccination data (present study) with post-vaccination data to gauge the impact of rotavirus vaccines in this region.

Ethical approval

This study was ethically approved by the Jamia Hamdard Ethics Committee (JHEC). Written informed consent was obtained from the parent or guardian on behalf of all the children enrolled in the study.

Authors' contributions

Shipra Gupta performed the experiments, analyzed the data and wrote the initial draft of the manuscript. Sanjeev Chaudhary helped in the clinical study design. Parvesh Bubber has read the initial draft of the manuscript. Pratima Ray is the PI, mentor and supervised the whole study including study design and final editing of the manuscript. All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors have no conflict of interest.

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