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A systematic review of rotavirus strain diversity in India, Bangladesh, and Pakistan

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ABSTRACT

Of the estimated half-million deaths from rotavirus globally each year, approximately one-third (N = 160,000 deaths) occur in the Indian subcontinent (defined as India, Bangladesh, and Pakistan). Two commercial vaccines are available for use and recommended by WHO, although the prohibitive vaccine price has limited their introduction into routine childhood immunization programs. New rotavirus vaccines are in late clinical development, including two advanced candidates in India. As significant shifts in rotavirus strain diversity have occurred in the past three decades and questions remain regarding whether strain replacement may occur following introduction of rotavirus vaccines, it is important to understand the temporal and regional strain diversity profile before vaccine introduction. We reviewed 33 peer-reviewed manuscripts from the Indian subcontinent and found that the most common G-types (G1–4) and P-types (P[4] and P[8]) globally accounted for three-fourths of all strains in the subcontinent. However, strains varied by region, and temporal analysis showed the decline of G3 and G4 in recent years and the emergence of G9 and G12. Our findings underscore the large diversity of rotavirus strains in the Indian subcontinent and highlight the need to conduct surveillance on a regional scale to better understand strain diversity before and after rotavirus vaccine introduction.

1. Introduction

Diarrheal disease is the second leading cause of under-five mortality worldwide [1,2]. Rotavirus is the most common cause of severe diarrheal disease in young children globally, attributing to >25 million clinic visits, an estimated 2 million hospitalizations, and approximately 527,000 deaths of children under 5 each year [3–5]. By the age of five, nearly every child in both developed and developing countries will contract rotavirus [5]; however, the great proportion of the burden of rotavirus is borne by young children in developing countries. In Africa and Asia, >75% of infants will have contracted their first serious rotavirus infection by 12 months of age and approximately 86% of the global mortality due to rotavirus occurs in these settings [4,5]. Furthermore, three countries in the Indian subcontinent (India, Bangladesh, and Pakistan) account for >30% (N = 160,000–200,000) of all rotavirus-related deaths worldwide [4,6–8]. This large burden of disease also creates an overwhelming economic burden on developing-country populations. For example, average expenditures per case treated in Vellore, India, came to 5.8% (large hospital) and 2.2% (small hospital) of the household annual income [8].

Symptomatic rotavirus presents itself most commonly as acute watery diarrhea, forceful vomiting, fever, and dehydration [9,10]. Rotavirus is highly contagious and resilient, and improvements to water and sanitation do not adequately prevent its transmission [5,11,12]. Malnutrition or co-infection with multiple enteric pathogens, common in developing countries, can further hinder effective rotavirus treatment, delay recovery, and lead to further sequelae, such as growth and developmental delays and susceptibility to re-infection. Therefore, prevention of rotavirus through immunization is considered a global priority to manage the disease [5,13].

Rotavirus vaccine development was influenced early by the observation that, due to the variety of strains circulating, a rotavirus vaccine needed to show heterotypic protection against the circulating strains to correctly assess the clinical efficacy [14]. The important antigenic characteristics of rotavirus strains are defined by two neutralizing antigens on the outer capsid – VP4 (a protease-sensitive protein protruding from the surface and labeled as the P-type) and VP7 (an outer capsid glycoprotein labeled as the G-type) [14]. These two antigens are encoded by separate genes and are able to segregate independently due to the segmented nature of the viral genome [15], resulting in theoretically >100

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combinations, which is demonstrated as new P- and G-types are discovered [16–20]. Early epidemiological evidence concluded that four rotavirus strains (P[8]G1, P[4]G2, P[8]G3, and P[8]G4) accounted for nearly 90% of all rotavirus strains circulating globally [21,22]. In the past decade, improved laboratory methods, including hybridization assays, oligonucleotide sequencing, and type specific reverse-transcriptase polymerase chain reaction (RT-PCR) primer kits, have enabled rotavirus surveillance efforts to examine more strains in greater detail, demonstrating far broader strain diversity in developing countries [17,18,22,23]. Thus, new rotavirus strains are discovered [17,19,20,23,24], novel P- and G-combinations are identified [16,17,19,20,23–25], and new emergent reassortant zoonotic strains are reported [26–28]. This prolific diversity is observed particularly in the subcontinent where a large number of studies have been conducted.

Two commercial rotavirus vaccines are currently available: Rotarix™ (GlaxoSmithKline Biologicals, Belgium), licensed in >100 countries worldwide, and RotaTeq® (Merck & Co., Inc., USA), licensed in approximately 90 countries worldwide. Both these commercial vaccines are pre-qualified by the World Health Organization (WHO) and are recommended for global use in all childhood immunization programs for the prevention of severe rotavirus disease [13]. In addition, several developing country manufacturers are developing a new pipeline of rotavirus vaccines [29,30]. Three of these candidate vaccines are currently in clinical development in India with different manufacturers, and one has completed Phase 2 immunogenicity studies [31,32]. The clinical development of any rotavirus vaccine for use in this region will require an understanding of the epidemiology and strain distribution to facilitate Phase 3 clinical studies and to act as a platform to eventually measure vaccine effectiveness.

Ongoing monitoring and review of strain diversity is thus necessary, not only to better understand strain diversity in specific regions, but also for the effective evaluation of vaccine efficacy against a multitude of strains, especially as national immunization policymakers respond to the WHO recommendation for the global use of rotavirus vaccine [13]. This systematic literature review of studies from India, Bangladesh, and Pakistan was conducted to establish a longitudinal description of rotavirus strain diversity and prevalence over three decades in a region that has high rotavirus mortality. Furthermore, the review should be useful for the planning of the Phase 3 studies with the new rotavirus vaccines that are in development by manufacturers in India, and for interpretation of the data that is generated.

2. Methods

2.1. Data abstraction

In order to measure patterns in rotavirus strain distribution, a systematic review of English literature published from January 1, 1983 to December 31, 2009 on human rotavirus strains in India, Bangladesh, and Pakistan was conducted using MOOSE (Meta-analysis of Observational Studies in Epidemiology Group) methodological guidelines [33]. Medline, ISI Web of Knowledge, and Proquest database were searched using the MeSH term "rotavirus" individually paired with "India," "Bangladesh," "Pakistan," "strain diversity," and "vaccine." Bibliographies of retrieved articles were reviewed for additional citations and experts in the field were consulted to ensure completeness of the search. Included in the review were all peer-reviewed studies that met the inclusion criteria of: (1) rotavirus-positive diarrhea samples, defined as 3+ watery stools, (2) samples originating from children aged 28 days to 6 years of age, (3) rotavirus genotype data from >20 samples using either ELISA, polyacrylamide gel electrophoresis (PAGE), or RT-PCR laboratory techniques, and (4) human studies using an observational study design (cohort, case-control, or cross-sectional). Neonatal strain data from both asymptomatic and symptomatic cases, which often pertained to single-strain nursery outbreaks [28,34] and insufficiently represented population-wide diversity, were excluded.

Pre-formatted data abstraction tables with demographic and epidemiological criteria (country, study site(s), region, laboratory methods, strains typed, novel strains, study length, study midpoint, maximum age of study sample, article appeared in previous literature review) were used. Type data was extracted by a single reviewer (MGM) and compiled in Microsoft Excel according to separate G- and P-types. In studies where G- and P-types were combined, results were separated to match the specifications of the database. The study midpoint was used to define four temporal categories (before 1994, 1995 to 1999, 2000 to 2004, 2005 to 2009) with the later date used when collection lasted an odd number of years.

2.2. Data analysis

Univariate and stratified analyses were conducted using SPSS version 18 and Microsoft Excel. Proportions reflect the frequency of each strain detected as the numerator and the total G or P samples tested across all studies as the denominator. Untypeable strains were excluded from the denominator due to inconsistencies in laboratory techniques and detection capabilities over time and across the literature. Unusual strains (G8, G10, G11, P[11], P[19]) were also excluded from the final analysis, but were cataloged for descriptive purposes. Regional divisions were based on the original author's definitions and include north (Delhi and Lucknow in India), east (Kolkata and Imphal in India; Dhaka/Matlab and Mymensingh in Bangladesh), south (Mysore, Bangalore, Vellore, Hyderabad, Chennai, and Trichy in India), and west (Pune and Mumbai in India; Karachi in Pakistan). The multiple categories combine studies completed at multiple sites without available disaggregated data.

3. Results

The search resulted in 161 relevant titles including information on human rotavirus strains in India, Bangladesh, and Pakistan. After reading abstracts and reviewing the full text, 33 studies (26 – India, 5 – Bangladesh, 2 – Pakistan) fulfilled the *a priori* selection criteria and were included in the meta-analysis (Table 1). Fourteen of the titles represented recent data not available in past reviews [18,37,63] and included studies using more advanced molecular methods for strain characterization. Both frontline urban hospitals and rural community health centers served as surveillance sites for collecting samples. Studies characterized both symptomatic and asymptomatic rotavirus cases from rainy and dry seasons.

A large variation in laboratory methods to detect rotavirus types was observed, with earlier studies (before 1994) relying principally on ELISA and PAGE, and later studies utilizing more advanced molecular RT-PCR techniques. Prior to 1994, two studies utilized PAGE, two utilized ELISA, and three utilized RT-PCR. From 1995 to 1999, 11 studies were published with 4 reporting PAGE techniques and 6 reporting RT-PCR; one study did not specify laboratory methods. The 15 studies from 2000 to 2009 relied entirely upon RT-PCR for genotyping, which represents the first time period that all results were fully based on RT-PCR techniques.

Overall, due to their later discovery in humans, 25 of the 33 studies (76%) did not use typing agents for detection of G12 while 11 of the earlier studies (33%) did not determine the G9 type. This

Table 1
Descriptive characteristics of studies with rotavirus serotype data meeting selection criteria for meta-analyses[a].

Study	Location (site, country)	Region	Data collection mid-point	Detection method(s)	Total G samples	Typed G samples[b]	G types not included
<1994							
Kelkar et al. [35]	Pune, India	West	1992	ELISA	205	107	G9, G12
Ramachandran et al. [36]	Multiple, India	Multiple	1993	RT-PCR	63	56	G12
Jain et al[c] [37]	Vellore, India	South	1984	PAGE/ELISA	46	32	G9, G12
Unicomb et al. [24]	Multiple, Bangladesh	East	1993	RT-PCR	2515	1420	G12
Nishio et al. [38]	Karachi, Pakistan	West	1994	ELISA/RT-PCR	70	70	G9, G12
Husain et al. [39]	Delhi, India	North	1991	RT-PCR	51	44	G9, G12
Aijaz et al. [40]	Mysore, India	South	1992	PAGE/ELISA	200	130	G9, G12
1995–1999							
Jain et al[c] [37]	Multiple, India	Multiple	1995	PAGE/ELISA	93	74	G9, G12
Jain et al[c] [37]	Chennai, India	South	1997	PAGE/ELISA	90	70	G9, G12
Jain et al. [41]	Multiple, India	Multiple	1997	RT-PCR	287	265	G12
Saravanan et al. [42]	Chennai, India	South	1997	PAGE/ELISA	118	118	G9, G12
Ananthan and Saravanan [43]	Chennai, India	South	1998	Not Specified	48	48	G12
Zade et al. [44]	Pune, India	West	1996	RT-PCR	90	90	G12
Kang et al. [45]	Vellore, India	South	1997	RT-PCR	145	120	G12
Das et al. [46]	Multiple, India	Multiple	1999	RT-PCR	159	130	None
Kang et al. [47]	Multiple, India	Multiple	1999	RT-PCR	82	68	G12
Chakravarti and Kumaria [48]	Delhi, India	North	1999	RT-PCR	100	66	G9, G12
Anand et al. [49]	Hyderabad, India	South	1999	PAGE	46	29	G9, G12
2000–2004							
Rahman et al. [50]	Multiple, Bangladesh	East	2002	RT-PCR	433	424	G9, G12
Rahman et al. [51]	Multiple, Bangladesh	East	2004	RT-PCR	468	468	None
Samajdar et al. [52]	Kolkata, India	East	2004	RT-PCR	147	147	None
Sharma et al. [20]	Sharma, India	North	2004	RT-PCR	465	437	None
Ramani et al. [19]	Vellore, India	South	2004	RT-PCR	462	361	None
Khetawat et al. [53]	Kolkata, India	East	2000	RT-PCR	140	124	G12
Das et al. [54]	Multiple, India	Multiple	2001	RT-PCR	126	111	G12
Bahl et al. [55]	Delhi, India	North	2001	RT-PCR	135	89	G12
Banerjee et al. [56]	Vellore, India	South	2003	RT-PCR	161	129	G12
2005–2009							
Dey et al. [57]	Dhaka, Bangladesh	East	2005	RT-PCR	307	307	G12
Paul et al. [58]	Mymensingh, Bangladesh	East	2005	RT-PCR	113	111	None
Kang et al. [59]	Multiple, India	Multiple	2006	RT-PCR	1404	1094	None
Samajdar et al. [60]	Kolkata, India	East	2006	RT-PCR	204	197	None
Mishra et al. [61]	Lucknow, India	North	2006	RT-PCR	79	75	G12
Qazi et al. [62]	Karachi, Pakistan	West	2006	RT-PCR	83	62	G12

[a] Disaggregated data by individuals years was used for temporal analysis if reported. Thus, Table 1 only provides a summary of each study and should not match study category totals in Table 4.
[b] Excludes untypeable strains.
[c] Original articles not available; data obtained from 2001 meta-analysis by Jain et al.

is reflected in the proportion of "untypeable" strains that were observed. When untyped strains were considered in the denominator of all tested specimens, 23.7% were untypeable prior to 2000. However, after 2000, when molecular typing methods were used and included primers for the G9 and G12 strains, the proportion of untypeable strains was reduced to 13.7%. A similar trend was noted in the results for the VP4 P-type, where 21.3% of strains could not be typed before 2000, compared to 16.3% after 2000, probably due to the wider range of primer sets used.

The 33 studies provide data on 9,153 rotavirus samples examined for the VP7 G-type, while 21 studies present results for 4,842 VP4 P-types. Among typeable G-samples ($n = 7703$) over the period covered in this review (1983–2009), the four most globally common types, G1 (31.4%), G2 (29.4%), G3 (3.6%), and G4 (13.8%), represented approximately 78% of total samples. During this same time period, G9 (11.2%), G-Mixed (6.9%), and G12 (3.7%) were also identified (Table 2). For the P-types, between 1983 and 2009, P[4] (29.3%) and P[8] (44.7%) represented approximately 75% of all the 4148 typeable P-strains, with P[6] (15.2%) and P-Mixed (10.8%) also present (Table 3). However, the percentages of uncommon G-types and mixed P-types reported may not accurately reflect the true proportions circulating in the population due to the number of untypeable strains showing current techniques.

3.1. Temporal distribution of strains

Overall, more strains were typed in the 9-year period from 2000 to 2009 (15 studies; 4149 strains) than in the last 17 years of the 20th century (18 studies; 2924 strains). Genotypes G1 or G2 were the most common strains across each time period; however, all strains varied over time (Table 4, Fig. 1) and non-G1 or -G2 strains rose to a proportion of ≥10% in only 5 separate seasons. G3 transitioned from the fourth most common strain in the time period before 1994 (9.6%) to the least common (1.2%) in the most recent period. On a relative scale, G4 underwent the most temporal change, decreasing from 31.3% of all strains in the period before 1994 to only 4.0% in 2005–2009 (Fig. 2). The decline in G3 and G4 strains was accompanied by an increase in G9 strains, which demonstrated peak prevalence of ∼15% from 2000 onward but had much lower detection rates in earlier periods. The presence of G12 typing and detection only emerged at the turn of the century, so now G12 strains constitute about ∼9.0% of these strains (262/2945), signaling steady transmission in the region. The number of strains with mixed G-types increased linearly over time by 7.2%, but probably reflects more sensitive molecular methods of detection (Table 4).

P-types remained more constant with P[4] and P[8] as the top two strains in each time period. P[6] types showed the most

Table 2
Number of rotavirus G-types collected by routine surveillance studies in Bangladesh, India, and Pakistan from 1983 to 2009 ($n = 9135$).

Ref.	Total G samples	Typed G samples[a]	G1	G2	G3	G4	G9	G12	G-Mixed	G UT/Other	G Novel (no.) [b]
Bangladesh											
[24]	2515	1420	324	423	77	540	56	NT[c]	NR[d]	1095	
[50]	433	424	194	65	NT	60	94	NT	11	9	
[51]	468	468	162	98	0	39	128	26	15	NR	G11 (3)
[57]	307	307	40	142	9	62	44	NT	10	NR	
[58]	113	111	24	61	0	1	18	4	3	2	
India											
[35]	205	107	15	49	1	9	NT	NT	33	98	
[36]	63	56	7	14	7	6	15	NT	7	7	
[37] [e]	46	32	15	3	2	12	NT	NT	NR	14	
[39]	51	44	17	13	5	4	NT	NT	5	7	G6 (3)
[40]	200	130	38	20	65	7	0	NT	NR	70	
[37] [e]	93	74	8	24	9	7	21	NT	5	19	
[37] [e]	90	70	10	34	5	16	NT	NT	5	20	
[41]	287	265	51	99	2	32	50	NT	31	22	G8 (1)
[42]	118	118	11	78	2	16	0	NT	11	NR	
[43]	48	48	7	33	1	0	0	NT	7	NR	
[44]	90	90	43	32	7	6	2	NT	NR	0	
[45]	145	120	50	24	1	30	5	NT	10	25	
[46]	159	130	61	38	0	20	0	0	11	29	
[47]	82	68	33	7	9	12	7	NT	NR	14	
[48]	100	66	31	12	18	5	NT	NT	NR	34	
[49]	46	29	16	0	0	8	NT	NT	5	17	
[52]	147	147	79	33	0	0	3	25	7	NR	
[20]	465	437	120	116	11	2	67	67	54	28	G2P[11] and G3P[11]
[19]	462	361	155	91	1	2	59	22	31	101	G8 (1) and G10 (69)
[53]	140	124	49	27	0	30	0	NT	18	16	
[54]	126	111	62	17	0	6	0	NT	26	15	G10 (15)
[55]	135	89	32	18	8	0	21	NT	10	46	
[56]	161	129	74	21	0	0	24	NT	10	32	
[59]	1404	1094	330	385	0	0	134	90	155	310	G10 (2)
[60]	204	197	82	65	0	0	20	28	2	7	
[61]	79	75	30	12	13	4	8	NT	8	4	
Pakistan											
[38]	70	70	30	10	0	30	NT	NT	NR	NR	
[62]	83	62	20	14	0	7	19	NT	2	21	
Total	9135	7073	2220 (31.4%)[f]	2078 (29.4%)[f]	253 (3.6%)[f]	973 (13.8%)[f]	795 (11.3%)[f]	262 (3.7%)[f]	492 (7.0%)[f]	2062	

[a] Excludes untypeable strains.
[b] Abstracted for descriptive analysis, not included in column totals.
[c] Not typed.
[d] Not reported.
[e] Original articles not available; data obtained from 2001 meta-analysis by Jain et al.
[f] Percent of total typeable strains; Total greater than 100.0% due to rounding.

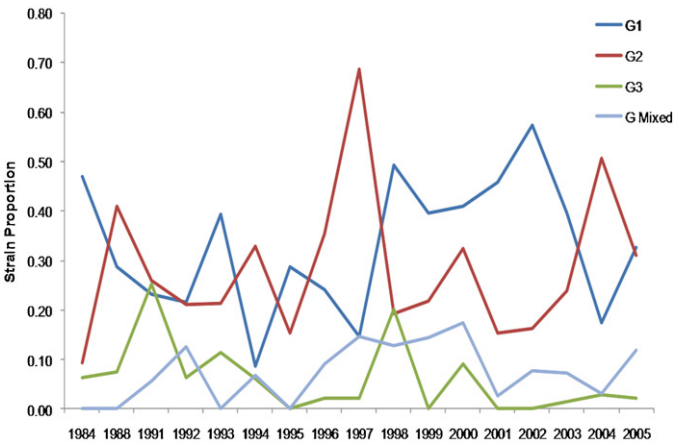

Fig. 1. Trends of rotavirus G1, G2, G3, and G mixed protein strain distribution from 33 surveillance studies in Bangladesh, India, and Pakistan (1983–2009).

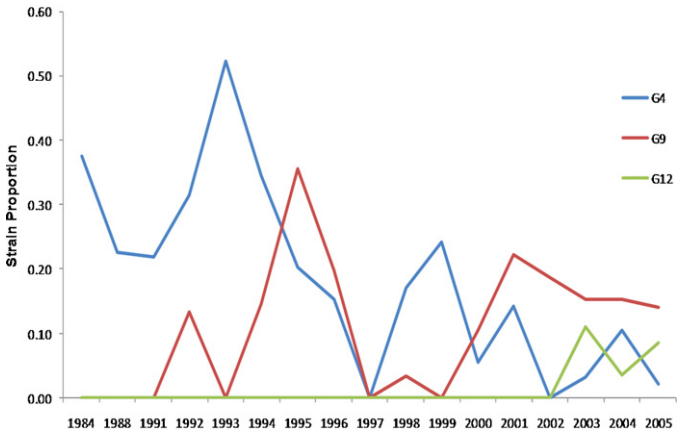

Fig. 2. Trends of rotavirus G4, G9, and G12 protein strain distribution from 33 surveillance studies in Bangladesh, India, and Pakistan (1983–2009).

Table 3
Number of rotavirus P-types collected by routine surveillance studies in Bangladesh, India, and Pakistan from 1983 to 2009 (n = 4842).

Ref	Total P samples	Typed P samples [a]	P[4]	P[6]	P[8]	P Mixed	P UT/Other[b]
Bangladesh							
[24]	236	236	45	55	136	NR[c]	
[50]	NT	NT[d]					
[51]	471	471	95	35	326	15	
[57]	307	284	119	1	149	15	23
[58]	113	111	56	22	16	17	2
India							
[35]	NT	NT					
[36]	62	55	13	27	8	7	7
[37] [e]	NT	NT					
[39]	57	43	14	4	23	2	14
[40]	NT	NT					
[37] [e]	91	72	23	28	16	5	19
[37] [e]	NT	NT					
[41]	277	253	63	88	69	33	24
[42]	NT	NT					
[43]	NT	NT					
[44]	89	89	38	11	40	0	
[45]	126	78	32	7	39	NR	48
[46]	139	120	35	8	51	26	19
[47]	82	52	19	2	31	NR	30
[48]	134	84	16	29	32	7	50
[49]	NT	0					
[52]	140	140	45	14	31	50	
[20]	457	428	90	104	162	72	29
[19]	NT	0					
[53]	NT	0					
[54]	126	99	19	61		19	27
[55]	NT	0					
[56]	166	100	28	3	65	4	66
[59]	1403	1093	375	80	483	155	310
[60]	204	204	64	15	111	14	
[61]	79	75	11	24	35	5	4
Pakistan							
[38]	NT	NT					
[62]	83	61	16	14	30	1	22
Total	4842	4148	1216 (29.3%)[f]	632 (15.2%)[f]	1853 (44.7%)[f]	447 (44.7%)[f]	694

[a] Excludes untypeable strains.
[b] UT = untypeable.
[c] Not reported.
[d] Not typed.
[e] Original articles not available; data obtained from 2001 meta-analysis by Jain et al.
[f] Percent of total typeable strains.

variation in prevalence (10.4%; frequency range 8.5–18.9%) and mixed infections also rose >7.4% between the earliest and latest time periods (Table 4).

Prior to 1995, 96.3% of all reported rotavirus strains matched antigens present in either RotaTeq® or Rotarix™ vaccines (G1–G4). However, by 2005–2009, the proportion of vaccine-matched strains circulating declined to 70.5%.

3.2. Regional distribution of strains

The south (1390 G-samples) and east (3340 G-samples) collectively totaled almost half of the review's sample size, with north, west, and multiple regional categories each contributing over 1000 G-samples (Table 5). G1 remained fairly constant across all regions, with the south identified as the only region in which G1 was not the predominant strain. Non G1- or G2-strains were found in proportions over10% among regions with >10 strains in any one season. G4 proved highly varied regionally, with only 1.7% in the north, 6.5% in the south, 7.0% in the west, and 21.9% in the east. G9 was found in proportions ≥10% in all but the west, while only G12 in the north had a proportion ≥10% (Fig. 2).

4. Discussion

This review of rotavirus strain diversity in India, Bangladesh, and Pakistan confirms that the Indian subcontinent maintains a more diverse rotavirus genotype portfolio than most regions in the world. Nevertheless, the most common G-types (G1–4) and P-types (P[4], P[8]) globally accounted for three-fourths of all strains over the total time period of almost three decades. Temporal analysis shows G3 and G4 clearly declining in recent years, while G9 and G12 emerge as increasingly dominant circulating strains. There has been a global trend of decline in the numbers of G3 and G4 strains, so this was not surprising; G9 and G12 strains have emerged into the human population as described elsewhere [23,24]. This study is also the first to systematically describe the introduction of G12 primers into laboratory testing and study methodologies in 2000 and document the subsequent growth in detection of G12 to 6.6% of strains by the 2005–2009 time period. Further, descriptive statistics of VP7-G1 demonstrate prevalence substantially different from the 72% to 82% found in North America, Europe, and Australia [22]. Far less variation appears in P-types throughout this review's temporal analysis, although a decreasing trend in P[6] appears evident.

This review adds the most current genotyping data to two earlier reviews on rotavirus strain diversity, both of which limited data to

Table 4
Temporal distribution of rotavirus G and P strains in Bangladesh, India, and Pakistan from 1983 to 2009; No (%).

Time period[a]	G1	G2	G3	G4	G9	G12	G-Mixed	G samples tested[b]	G UT[c]	G Novel	P[4]	P[6]	P[8]	P-Mixed	P samples tested[b]	P UT[c]
≤1994	439(25.7)	507(29.7)	164(9.6)	534(31.3)	16(0.9)	NT	45(2.6)	1705	1279	3	67(28.8)	44(18.9)	113(48.5)	9(3.9)	233	21
1995–1999	324(26.6)	398(32.6)	47(3.9)	226(18.5)	139(11.4)	NT	85(7.0)	1219	192	1	223(26.7)	210(25.1)	332(39.7)	71(8.5)	836	190
2000–2004	931(40.4)	494(21.5)	20(0.9)	139(6.0)	397(17.2)	140(6.1)	182(7.9)	2303	247	88[c]	285(22.8)	222(17.7)	584(46.7)	160(12.8)	1251	122
2005–2009	526(28.5)	679(36.8)	22(1.2)	74(4.0)	243(13.2)	122(6.6)	180(9.8)	1846	344	2	641(35.1)	156(8.5)	824(45.1)	207(11.3)	1828	361

[d]Ramani et al. [20] reported 70 novel strains (69 – G10, 1 – G8).

[a] Midpoint of disaggregated strain data from each included study.

[b] Excludes untypeable and novel strains.

[c] UT = Untypeable.

Table 5
Regional distribution of rotavirus G and P strains in Bangladesh, India and Pakistan from 1983 to 2009; No (%).

Region	G1	G2	G3	G4	G9	G12	G-Mixed	G samples tested [a]	G UT[b]	P[4]	P[6]	P[8]	P-Mixed	P samples tested [a]	P UT[b]
North	281(31.5)	226(25.4)	55(6.2)	15(1.7)	122(13.7)	89(10.0)	103(11.6)	891	171	180(22.2)	189(23.3)	329(40.6)	112(13.8)	810	149
South	428(30.8)	475(34.2)	77(5.5)	91(6.5)	168(12.1)	42(3.0)	109(7.8)	1390	401	228(43.0)	22(4.2)	246(46.4)	34(6.4)	530	236
East	1008(30.2)	938(28.1)	86(2.6)	732(21.9)	369(11.0)	85(2.5)	122(3.7)	3340	1166	451(28.4)	153(9.6)	817(51.4)	167(10.5)	1588	62
West	281(37.6)	240(32.1)	8(1.1)	52(7.0)	43(5.7)	46(6.1)	78(10.4)	748	218	185(32.5)	54(9.5)	286(50.3)	44(7.7)	569	121
Multiple	222(31.5)	199(28.3)	27(3.8)	83(11.8)	93(13.2)	NT[c]	103(11.4)	704	106	172(26.4)	214(32.9)	175(26.9)	90(13.8)	651	126
Total	2220	2078	253	973	795	262	492	7073	2062	1216	632	1853	447	4148	694

[a] Excludes untypeable and novel strains.

[b] UT = Untypeable.

[c] NT = Not tested.

India only. A report by Jain et al., depicted G1 (16%), G2 (24%), G3 (15%), G4 (10%), G9 (6%), and G-Mixed (8%) in circulation between 1983 and 1997, which aligns with our analysis from this time period [35]. With data up to 2004, Kang et al. in 2005 highlighted a 9% increase in G9 from previous periods coupled with a 4% decrease in G3 [18].

4.1. Emergent strains

The emergence of G9 in Bangladesh and India occurred a decade after it was first discovered in Philadelphia, Pennsylvania, USA, in 1983/1984. G9 strains were first identified as increasing in prevalence in Bangladesh in 1995 [24] and have subsequently become the third most common strain globally. G9 strains appeared about the same time in India [34]. Interestingly, in India, G9P[11] was first detected in a neonatal outbreak. This strain was most likely replaced with G9P[6] when it reassorted with common P[6] neonatal strains, eventually reassorting with the more virulent human P[8] strains circulating in the community and multiplied under a new lineage as G9P[8], the most common G–P combination across India [34]. This review shows that G9 now holds the position of India's third most prominent genotype.

In the past 16 years, VP7 G9 has been observed in combination with an unusual number of P-types, both VP6 subgroups I and II and both long and short RNA electropherotypes. This has been postulated as putative evidence of a distinct biological advantage over other common strains to reassort with circulating strains [27]. Recently, oligonucleotide sequencing of a G9P[6] strain from Kolkata (strain ISO-5) detected high similarity to the porcine P[6] gene, evidence of either a whole virus transmission or an alternative zoonotic reassortment event with human rotaviruses [27].

VP7 G12 was first characterized serologically in the Philippines in 1987 and was initially limited in circulation among humans. However, G12, in association with P[4], P[6], and P[8], has recently emerged in India and Bangladesh, paralleling its widespread global emergence in 2005 [64,65]. First detected from a routine rotavirus surveillance study in Kolkata in 2001 [38], G12 rapidly spread across the country and by 2007 appeared consistently in large-scale, multicenter surveillance across India [65]. The incorporation of G12 primers into RT-PCR testing kits since 2000 has helped establish prevalence data for G12 strains in India. Continued surveillance will be necessary to document an expected trend of expansion and reassortment in coming years.

Nucleotide sequence of the VP7 gene from a 2005 community cohort study found 13 G12 strains with homology to the G12 Kolkata ISO-5 strain (97–99% nucleotide level) as compared to the G12 L26 prototype strain lineage I or lineage II (89–90% nucleotide level) of the phylogenetic tree [66]. These results suggest a distinctly native G12 lineage III strain in India [66]. However, it appears the Indian G12 lineage is continually evolving, with multiple reassortment events and several new gene constellations. A second study of all 11 genes from G12 strains in Bangladesh, Belgium, the Philippines, and Thailand characterized vast nucleotide variability from the original Kolkata strain [23]. Such reassortment ability is hypothesized to improve the ability of G12 to propagate within the human host and potentially launch it on a similar path of rapid transmission as G9 [23].

4.2. Unusual strains

Historically, Asia has birthed many new rotavirus strains, including the G10P[11] in 1993, a likely human-porcine reassortment (P[19]) in the early 1990s, and, most recently, G11P[25] [41,51,64]. Oligonucleotide analysis of G11P[25] from Bangladesh found the VP7 gene to share the most similarity (95% amino acid identity, 87–91% nucleotide identity) with the porcine G11 rotavirus strains; however, the VP4 genotype presented low similarity (54–71% nucleotide identity and 52–76% amino acid sequence identity) to the porcine isolate and thus likely indicates a new human-animal reassortment virus named Dhaka6 [64]. Dhaka6 has subsequently been identified in Vellore neonates with 98% (VP7) and <96% (VP4) nucleotide similarity [16].

Beyond the common G1, G2, G3, G4, and G9 strains, 14% more unusual strains appear in Asia as compared to the US and Australia [22]. Mixed infections, along with human-animal reassortments, sustain an environment fit for such cases. Unusual G-types (G6 and G8) and strains (G3P[11] and G9P[10]) have been described through multiplex RT-PCR, nucleotide sequencing, and hybridization assay, highlighting the wide genetic and antigenic diversity of strains circulating in the region [22].

Such variation evokes the need for continued surveillance to serve two important functions. First, as new rotavirus vaccines are currently in development, it is important to assess and consider the strain variability in the design of the new vaccine candidates and in the clinical evaluation of the vaccines in regions with high strain diversity. Two philosophies exist regarding the need for neutralizing antigens in the vaccine construct to elicit specific neutralizing antibodies in the host. One approach utilizes a monovalent human rotavirus strain, such as the GSK rotavirus vaccine and one candidate being developed in India [31]. Another approach emphasizes the need to generate neutralizing antibodies by including several G and P types in the vaccine construct, similar to the Merck rotavirus vaccine. There has also been the suggestion that a "designer" vaccine could be developed for specific regions based on the local rotavirus strain diversity [30].

Second, it is crucial to have ongoing surveillance to measure impact once vaccines have been introduced and to assess the potential impact of large-scale vaccination programs on strain diversity and circulation. In this regard, it should be noted that natural variation of rotavirus strains appears high in this region even prior to vaccine introduction and some variation in time and region is to be expected.

Study limitations include over-interpretation from a relatively small number of samples (<10,000), variations in sample populations and collection site (hospitalized vs. non-hospitalized cases), and use of different assays for strain detection; the last limitation is particularly applicable to the period prior to 1995 when molecular methods for typing were not widely deployed. No formal quality assessment was conducted beyond selection criteria requirements. Finally, although this review expands the knowledge of strain diversity in the Indian subcontinent to countries outside of India, limited data were available from Pakistan in particular.

Overall, these results reflect the ubiquitous nature of strain diversity both in terms of proportional distribution, emergence of unusual lineages, and presence of recombinant strains over the past three decades. These results also show differences in strains between regions within the Indian subcontinent during the same time period. Taken collectively, this systematic review and meta-analysis underscores the large diversity of rotavirus strains in this region and the need to conduct surveillance studies on a regional scale to better understand strain diversity before and after rotavirus vaccine introduction. The nature of which mechanisms drive strain diversity and molecular evolution have been postulated, and include antigenic drift and antigenic shift, as well as reassortment events [67]. One intriguing question is whether the wide spread use of rotavirus vaccination and the ensuing population immune pressure might drive molecular evolution of rotavirus strains. Given the enormous rotavirus strains genetic diversity in the Indian subcontinent, the huge disease burden and the future introduction of rotavirus vaccines in the region, a strong platform of surveillance and strain determination would enable this analysis as vaccines are rolled out.

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