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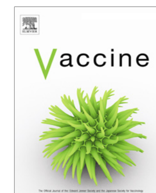

Genotype distribution of Group A rotavirus from southern India, 2005–2016

Sudhir Babji [a,*], Rajesh Arumugam [a], R. Priyahemavathy [a], Archana Sriraman [a], Anuradha Sarvanabhavan [a], Punithavathy Manickavasagam [a], Anna Simon [b], Indira Aggarwal [b], Prabhakar D. Moses [a], Rashmi Arora [c], Gagandeep Kang [a]

[a] Division of Gastrointestinal Sciences, Christian Medical College, Vellore, India
[b] Department of Child Health, Christian Medical College, Vellore, India
[c] Epidemiology and Communicable Diseases Division, Indian Council of Medical Research, New Delhi, India

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ABSTRACT

Diarrheal disease due to Group A rotaviruses remain a leading cause of mortality and morbidity in the less developed parts of the world. India has started a phased roll out of rotavirus vaccine in the national immunization program. This analysis summarizes the rotavirus genotype strain distribution pre-vaccine introduction in Vellore, India from December 2005 to June 2016. Rotavirus was responsible for 32% of all diarrheal admission to the hospital. G2P[4] was the predominant strain in the initial years and was gradually replaced by G1P[8]. The emergence of G9P[4] replacing G9P[8], and the detection of G12 strains over several years were documented. There was no clear seasonality of disease. These data form the baseline to monitor genotype distribution post-vaccine introduction in Tamil Nadu.
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1. Introduction

Group A rotaviruses are the leading cause of acute gastroenteritis related morbidity and mortality in children under 5 years of age, in the developing world. India is a significant contributor to the worldwide rotavirus burden with an estimated 47100 rotavirus deaths occurring in 2013, accounting for 22% of the world's rotavirus related mortality in children <5 years of age [1]. Rotavirus diarrhea costs India approximately INR 4.9 billion annually in hospitalizations and an additional INR 5.8 billion on outpatient visits [2].

Rotaviruses are non-enveloped, icosahedral, triple layered particles, with the outer capsid layer consisting of VP7, a glycoprotein (G) and VP4, the protease-sensitive (P) protein. Till date 27 G and 35 P types been described of which 73 G and P combinations have been detected in humans, with G1P[8], G2P[4],G3P[8], G4P[8] and G9P[8] being the most commonly detected genotypes [3]. The first large scale rotavirus strain surveillance network in India ran from 2005 to 2009, involving multiple centres across the country [4]. This was followed up with an expanded program from June 2012 [5]. The main aim of these surveillance programs was to measure the rotavirus disease burden across India, by monitoring hospital admissions due to rotavirus gastroenteritis and to understand the rotavirus genotype distribution across the country.

At present, two globally licensed vaccines (Rotarix®, GlaxoSmithKline Biologicals and RotaTeq®, Merck & Co., Inc) against rotavirus gastroenteritis are available in the private sector in India. These were recommended for use by the Indian Academy of Pediatrics (IAP) in 2013 [6] following bridging studies in India [7]. India also licensed an indigenously developed vaccine (ROTAVAC®, Bharat Biotech Ltd.) in 2014 [8], which is being introduced in a phased manner in the national immunization program and the IAP has updated its recommendations to include this vaccine. Compared to the developed world, these vaccines have lower efficacy in developing countries [9].

With the increased availability and use of these licensed vaccines, it is important to monitor the circulating rotavirus genotypes to detect changes or the emergence of new strains. This report describes the rotavirus strain distribution from children <5 years of age admitted with acute gastroenteritis at the Christian Medical College (CMC), Vellore, a tertiary care center in South India from December 2005 to June 2016.

2. Material and methods

The Wellcome Trust Research Laboratory (WTRL) at CMC was part of the Indian Rotavirus Strain Surveillance Network supported by Indian Council of Medical Research (ICMR) from December 2005 to June 2009 [10]. The WTRL continued the surveillance from July 2009 to June 2012 at three centers - Trichy, Vellore (July 2009 to

* Corresponding author.
E-mail address: sudhirbabji@cmcvellore.ac.in (S. Babji).

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May 2011) and Delhi [11]. Subsequently, the expanded National Rotavirus Surveillance Network (NRSN) initiated in September 2012 to June 2016. Uniform criteria for recruitment, stool sample collection, laboratory detection and genotype characterization protocols were used at all sites. Here we report the data only from CMC, Vellore describing the genotype variation over the entire time period. All studies were approved by the institutional review board (IRB) at CMC, Vellore.

2.1. Laboratory methods

Stool samples were collected from children <5 years of age hospitalized with acute gastroenteritis [10]. Samples were tested for the presence of rotavirus using a commercial antigen detection ELISA kit (EIA; Rota IDEIA; Dako for samples collected between December 2005 to June 2009 and Premier™ Rotaclone®, Meridian Biosciences from July 2009 to June 2016) following the kit instructions [4,11]. Additional internal control with a predefined range was included in all runs, and the runs were repeated if any of the controls (kit provided as well as the internal control) did not fall in the expected range.

Genotyping was performed on all ELISA positive samples. RNA was extracted from using Trizol (Invitrogen Life Technologies) from the samples collected from 2005 to 2009 and QIAamp Viral RNA Mini Kit from samples collected from 2009 to 2016. RNA denaturation was carried out at 97 °C for 5 min, followed by chilling at 4 °C for 2 min and complementary DNA was synthesized using random primers (Pd(N)6 hexamers; Pharmacia Biotech) and 400 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) and was used as template for VP7 and VP4 typing in hemi-nested multiplex PCRs using published oligonucleotide primers and protocols. PCRs to detect VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12 and VP4 genotypes P[4], P[6], P[8], P[9], P[10], and P[11] were performed [4].

ELISA positive samples for which both G and P type, could not be determined by the above method were confirmed to be rotavirus positive by PCR to detect the VP6 gene. If VP6 positive, these samples were further characterized by using alternate extraction methods, additional primer sets and sequencing as described previously [12,13]. These methods were also used for samples which were partially typed on first testing.

3. Results

For this analysis, 3036 samples collected at CMC, Vellore between December 2005 till June 2016 were included. Rotavirus was detected in approximately 32% (971/3036) of samples (Table 1). The median (IQR) age of positivity was 10 months (6,15). Of the positives, 62% were male at a median (IQR) age of 10 months (7,15). There was no seasonal difference in rotavirus positivity rates over the study period.

Of the 971 samples positive for rotavirus, 798 (82%) samples could be G typed and 739 (76.1%) could be P typed at first testing. Of the samples which could not be G and P typed (159/971, 22%), 118 (12%) were found to be VP6 negative, thus indicating false positive ELISA results. Thus 4.8% (41/853) remained untyped. There was no clustering seen of the untyped or partially typed samples over the study period.

Both G and P typing could be determined for 684 (80.2%) (Table 2). G1P[8] was the most commonly identified genotype (50%) followed by G2P[4] (22.4%) (Table 2). The other commonly identified genotypes were G12 in combination with either P[8] or P[6] and G9 in combination with either P[8] or P[4]. The other less commonly identified genotypes were G3 and G10. In December 2005, G9P[8] was the most commonly detected genotype. During 2006–2007, G2P[4] was the most commonly identified genotype but from 2008 onwards G1P[8] was the predominant genotype identified. From January 2016 to June 2016, G9P[4] was the most frequently detected genotype (14/33, 42%) (Fig. 1).

4. Discussion

This analysis presents the genotype distribution from Vellore, South India from December 2005 to June 2016. In 2011, samples were collected from January to May, because the ICMR surveillance ended and hence the number of samples for that year is low. G2P [4] was the predominant genotype identified until 2007 when it was replaced by G1P[8] which remained the most common genotype subsequently. This period also saw the emergence of G12 and G9 strains in Vellore. As described previously, G12 strains were identified for the first time in Vellore from stool samples collected from a birth cohort study to understand the natural history of rotavirus [14]. This led to a modification of the genotyping protocol with addition of primers to detect G12 [15], following which detections increased and continued to be reported in combination with P[8] and P[6], with only one detection with a P[4].

G9 strains identified initially were associated with P[8] and have been gradually replaced by P[4], with G9P[4] becoming the predominant genotype in 2016. This reflects the changing epidemiology of G9 strain circulation as also reported by others [16].

The ELISA false positive rate was 12% over the study period. These samples could be false positive due to presence of antigen but degraded nucleic acid in the stool sample. The presence of PCR inhibitors might also result in PCR negatives from ELISA positive specimens.

Rotavirus genotypes vary in time and space. Surveillance from Brazil clearly demonstrates predominant genotypes varying with time, showing an increase in G9P[8] and decrease in G2P[4] detection rate from 26% in 1982–1995 to 2% during 1996–2005 [17]. Data published from Kenya also shows an increase in detection of G8P[4] strains from 2% in 2002 to 13% in 2004 [18]. The data from Brazil also shows region specific genotype predominance, much like India where G12 strains are predominantly detected from the northern and western regions and G1 strains from South India [10]. This geographic difference in detection of predominant genotypes has been reported from New Zealand as well, where G1 was most commonly identified in the North Island as compared to G4 which was predominant in the South Island [19].

The availability and use of licensed vaccines may also influence the genotype distribution. India has introduced rotavirus vaccine in a phased manner starting in one state in December 2015. At present, 9 states have been covered with further expansion planned to cover nearly half the Indian birth cohort by mid-2018 [20].Brazil, which uses Rotarix® has reported the predominant circulation of G2 strains from 2006-2010 with increased G9 circulation in 2011, replaced by G12s in 2014 [21]. The introduction and use of

Table 1
Overall rotavirus percentage positivity over the study period.

Year	2005*	2006	2007	2008	2009	2010	2011*	2012	2013	2014	2015	2016*	Overall
% positive (Total n)	48 (52)	39 (398)	31 (287)	45 (279)	29 (251)	30 (156)	11 (19)	25 (309)	28 (376)	32 (358)	31 (359)	22 (189)	32 (3036)

* For the year 2005 samples included were collected in December, for 2011 January to May and for 2016, January to June.

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Table 2
Combination of G and P types of rotavirus strains genotyped in Vellore from 2005-2016.

Genotype		Number of strains P[4]	P[6]	P[8]	Other P types	P untyped	Mixed P types	Total Number	Percentage (%)
G1		4	10	342	0	27	5	388	45
G2		153	1	7	0	16	0	177	21
G9		36	4	65	0	21	2	128	15
G12		1	17	20	0	3	0	41	5
Other G types		0	0	4	12	1	0	17	2
G untyped		7	1	6	0	41	0	55	6
Mixed G types		16	3	15	0	5	8	47	6
Total									
	Number	217	36	459	12	114	15	853	100
	Percentage (%)	25	4	54	1	13	2	100	

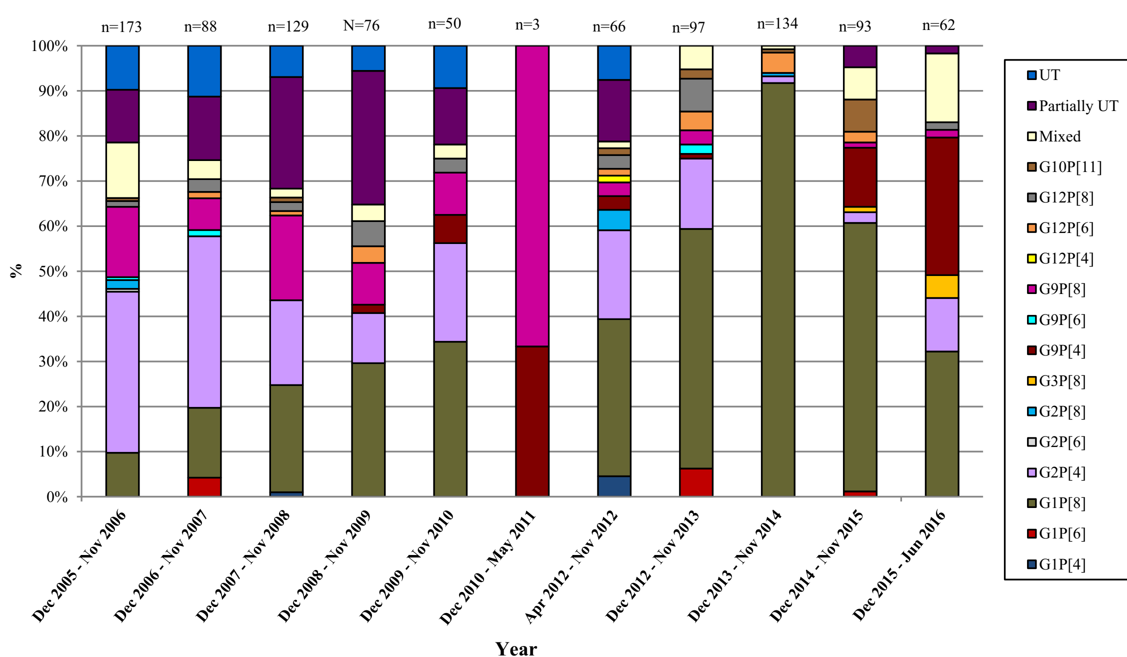

Fig. 1. Proportion of G and P type strains detected in Vellore from December 2005 to June 2016.

Rotateq® in USA and regions in Australia was followed by an emergence of G3 strains [22,23]. Additionally, following Rotarix® use in parts of Australia an emergence of G2 strains was reported [22]. In Belgium, after the introduction of Rotarix® in 2006, G2 prevalence remained consistently above 30%, a trend which continued in the subsequent years as well, when compared to pre-vaccine introduction, when G2 was detected in <20% of the genotyped strains [24].

The availability and use of the globally licensed rotavirus vaccines as well as the roll out of the indigenously developed vaccine at the national level makes the Indian rotavirus surveillance system even more relevant to be able to monitor disease and the strain distribution. Tamil Nadu, having generated genotyping data over nearly two decades, is in a unique position to now monitor the genotype distribution post vaccine introduction.

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