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RAPID COMMUNICATION

Apparent extinction of non-G2 rotavirus strains from circulation in Recife, Brazil, after the introduction of rotavirus vaccine

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Abstract The introduction of a G1P[8] rotavirus vaccine in Recife, Brazil, caused a decrease in rotavirus detection from 27% (March–May, 2006) to 5.0% (March–May, 2007), with all strains becoming G2, against which less protection had been predicted.

Rotavirus is the most important cause of severe, acute gastroenteritis in infants and young children, with a high mortality in developing countries [4, 11]. With highly efficacious, live, oral rotavirus vaccines available [13, 14], accelerated introduction of rotavirus vaccines into a universal childhood immunization program is a priority for international collaborative initiatives such as the Global Alliance for Vaccines and Immunization [12].

Rotavirus has two viral neutralization antigens, VP7 and VP4; the serotype defined by VP7 is termed the G (for glycoprotein) serotype, and that defined by VP4 is termed the P (for protease-sensitive) serotype [5]. Knowledge of the distribution of G and P serotypes, including the detection of emerging serotypes, is critical to rotavirus vaccine programs [8].

On 6 March 2006, Brazil, a country with the largest birth cohort in Latin America, at approximately 4 million, initiated universal immunization of infants with a serotype G1P[8] human rotavirus vaccine (Rotarix, GlaxoSmithKline Biologicals, Belgium). This vaccine has shown an excellent protective efficacy against non-G2 strains in large phase III clinical trials, performed mostly in Latin American countries [13], but it appeared less efficacious against G2 rotavirus strains, with which the vaccine strain shares neither the G nor the P serotype [4]. Therefore, this study was undertaken to evaluate the effect of this national rotavirus immunization program on the circulation of rotavirus strains over a 15-month period starting concurrently with the vaccine introduction among children in Recife, one of the largest cities in the North-East region of Brazil, with a population of approximately 1.5 million.

Fecal specimens were collected from children under 5 years of age who presented with acute gastroenteritis (defined as three or more passages of liquid or semi-liquid stool within the preceding 24 h) to the emergency services of a 500-bed teaching hospital in Recife (Instituto Materno Infantil Professor Fernando Figueira), Pernambuco, Brazil, during a 15-month period between March 2006 and May 2007.

Rotavirus antigen was detected by using an enzyme-linked immunosorbent assay (Rotaclone, Meridian

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Bioscience, Inc., Cincinnati, OH, USA). Following extraction of genomic RNA [6], rotavirus G and P types were determined with a hemi-nested multiplex RT-PCR, using consensus and type-specific primers as described previously [6, 7].

Genomic RNA extracted from stool specimens was separated by electrophoresis on a 10% polyacrylamide gel in an Amersham SE600 Ruby apparatus, followed by staining with silver nitrate or ethidium bromide.

To ascertain the identity of electropherotypes, nine G2P[4] rotavirus specimens with insufficient amounts for repeated electrophoresis experiments were culture-adapted using MA104 cells essentially according to the method described by Kutsuzawa et al. [9]. Cultured viruses, including those designated Rec03/06 and Rec127/07 (see later), were also used for the extraction of the genomic RNA, from which the *VP7* genes were then amplified by reverse-transcription PCR with primers Beg9 and End9 [7]. The PCR products were sequenced by the Genomic Research Center, Shimadzu Corporation, Kyoto, Japan.

Group A rotavirus antigen was detected in 70 (15%) out of 470 fecal specimens. Characterization of these strains into G and P types and RNA electropherotype is summarized in Table 1. Of 70 rotavirus-positive samples, we were able to assign 87% to G types and P types. The most prevalent G type was G2 (56%), of which 92% were of P[4] specificity. The second most prevalent G type was G9 (23%), all of which were typed as P[8]. There were three mixed infections with more than one G type. All G2 strains had short RNA patterns which were classified into ten electropherotypes (data not shown). Assuming that each electropherotype represents a single rotavirus strain, there were two dominant G2P[4] strains, one (Rec03/06) circulating in the first 6 months and the other (Rec127/07) circulating in the last 4 months. Electropherotypes carried by Rec03/06 and Rec127/07 were similar to each other, and there was only a minimal level of difference in the migration of segment 8 upon co-electrophoresis. In fact, there were eight substitutions (99.2% nucleotide identity), causing only two amino acid changes (residues 17 and 72) found between the VP7 genome segments of Rec03/06 and Rec127/07, both of which were located outside of the antigenic regions of the VP7 protein.

To evaluate the effect of the universal immunization program in Brazilian infants with Rotarix, a G1P[8] human rotavirus vaccine, starting in March, 2006, we compared the detection rates of rotavirus diarrhea in the first three months of the study period (between March and May, 2006) with those in the last three months (between March and May, 2007). Both 3-month periods included the rotavirus peak season in Recife, Brazil.

First, we observed a significant reduction in the detection rate of rotavirus from 27% (45/166) in the first 3 months to 5.0% (11/221) in the last 3 months ($p = 0.00013$ by Fisher's exact test). Since the coverage for rotavirus vaccine had reached 49% in the vicinity of Recife at the end of 2006 (http://www.datasus.gov.br), it is conceivable that the reduction in rotavirus detection rate among diarrhea cases was due to the effect of the rotavirus vaccine.

Second, among those specimens that were found to be rotavirus-positive, 47% (21/45) were typed as G2 in the first 3 months, whereas 100% (11/11) were typed as G2 in

Table 1 G and P serotype/genotype combinations and RNA-PAGE patterns of Rotaclone-positve rotavirus strains detected in stool specimens collected from children with acute diarrhea in Recife, Brazil, during the period between March 2006 and May 2007

G and P type combination		PAGE						
		Long[a]	Short[a]	Long + short	Positive[b]	Negative	Not done	Total (%)
G1	P[8]	2			1	1		4 (5.7)
G1	P[4]		2					2 (2.9)
G2	P[4]		32		3	1		36 (51.4)
G2	P[NT]		2			1		3 (4.3)
G9	P[8]	8			4	3	1	16 (22.8)
GNT	P[4]				1			1 (1.4)
GNT	P[8]					2		2 (2.9)
GNT	P[NT]		1			1	1	3 (4.3)
G1 + G9	P[8]	1						1 (1.4)
G2 + G9	P[4]			2				2 (2.9)
Total		11	37	2	9	9	2	70 (100)

[a] A long pattern is defined as the one having faster-moving tenth and 11th genome segments, and a short pattern is defined as the one having slower-moving tenth and 11th genome segments

[b] Rotavirus-specific RNA bands were visible, but determination of RNA patterns was difficult

the last 3 months ($p = 0.0013$ by Fisher's exact test). Thus, there was a complete predominance of G2 strains over the other serotypes 1 year after vaccine introduction. However, G2 strains were on an increasing trend, because nearly half of the rotavirus strains were of serotype G2 even at the onset of the rotavirus vaccine program, and G2 strains were detected in only 7% of cases in the study performed at the same hospital 1 year before the introduction of rotavirus vaccine (May 2004–April 2005) [10].

In this regard, studies conducted during the last decade in Latin America, including Brazil, have described the great diversity of rotavirus strains [3]. The relative frequency of G2 was reported to be 30% on average. Specifically, serotype G2 strains were the most prevalent, and they accounted for 34% of rotavirus-positive specimens in Rio de Janeiro from 1996 to 1998 [1]. During the same period it was also documented that G2P[4] strains were the most prevalent in large Argentinean cities, and they accounted for, on average, 30% of rotavirus-positive specimens, and as much as 36% was observed in one of the cities [2]. Nevertheless, the degree of predominance of G2 strains we observed in Recife over this 15-month period is unusually high. Thus, it seems likely that such predominance of G2 strains in circulation was due to the effect of the vaccine strain used in Brazil, which may be less efficacious against G2 strains [13], although it could still be within the random fluctuation of rotavirus genotypes.

While this study is among the first to evaluate the impact of rotavirus vaccine in Brazil, it is an ecological study and has limitations. In particular, we did not compare the incidence of rotavirus-associated diarrhea between those who were vaccinated and those who were not vaccinated, thus it was not possible to accurately evaluate the effectiveness of the rotavirus vaccine in the community. Despite such limitations, the observations we have made are suggestive of the impact of the rotavirus vaccine on the circulation of rotavirus strains in the community.

In conclusion, we offer a hypothesis that, while rotaviruses of serotype G2 were already increasing in prevalence, the introduction of the monovalent G1P[8] vaccine into the childhood immunization schedule created conditions in which serotype G2P[4] strains had a selective advantage over non-G2 strains, which were mostly of P[8], and that non-G2 strains have apparently been removed from circulation in the community. This hypothesis is worthy of testing by continuing surveillance in Recife for a longer period of time as well as carrying out post-licensure vaccine effectiveness studies.

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Molecular Epidemiology of Rotavirus Diarrhea among Children in Saudi Arabia: First Detection of G9 and G12 Strains▽

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In anticipation of rotavirus vaccine introduction in Saudi Arabia, this study was undertaken to determine the distribution of the G and P genotypes of rotaviruses in order to examine whether there was any emerging serotype or unusual strain circulating in children in Saudi Arabia. Of 984 stool specimens collected between 17 April 2004 and 16 April 2005, rotavirus was detected by an enzyme-linked immunosorbent assay in 187 (19%) diarrheal children less than 5 years of age. Of these, 160 (86%) were classified into G and P genotypes as follows: G1P[8] (44%), G2P[4] (20%), G9P[8] (11%), G12P[8] (4%), and G3P[8] (4%). RNA polyacrylamide gel electrophoresis identified 94 (50%) specimens as long RNA patterns, 30 (16%) specimens as short RNA patterns, and 1 mixed infection. Only a single long RNA electropherotype was identified for seven specimens containing G12P[8] rotavirus. RNA-RNA hybridization demonstrated that the G12P[8] strains were similar in their genomic constellation to locally cocirculating strains and to a Nepalese G12P[8] strain. The Saudi Arabian G12 VP7 gene had a 99% nucleotide sequence identity with Nepalese and Indian G12 VP7 genes and belonged to the third lineage. This study is the first to describe the distribution of rotavirus G and P types and also the first to identify G9P[8] and G12P[8] strains in the country.

Rotavirus is the single most important etiological agent causing severe diarrhea in infants and young children in both developing and developed countries (6). While virtually all children experience rotavirus infection by the age of 3 to 5 years wherever they live (12), the majority of an estimated 527,000 rotavirus-associated deaths occur in developing countries (41). Two, live, orally administrable rotavirus vaccines are currently licensed in many countries after they had gone through large-scale safety and efficacy trials, each involving more than 60,000 children in both developing and developed countries (32, 40).

The genome of rotavirus comprises 11 segments of double-stranded RNA, which are encased within a triple-layered capsid. The outermost capsid of the virion is composed of two independent neutralization antigens, VP7 and VP4, which define the G serotype and the P serotype, respectively (6). Since molecular assays are more routinely used than serological assays to determine these serotypes, the term genotype is more often used to define the VP7 and the VP4 specificity of a given strain. Since G and P type-specific immunity is believed to play a role in protection against disease, the epidemiology of G and P serotypes (and genotypes) of circulating strains forms a critical knowledge base for the development and implementation of rotavirus vaccines (19).

Rotavirus nonstructural protein NSP4, encoded by genome segment 10, has been shown to function as a viral enterotoxin (28). Extensive sequence analysis has shown that human and animal NSP4 genes fall into five genetic groups (A to E), and strains tend to cluster within genotypes according to their species of origin (4, 7, 15–17). Thus, examination of the NSP4 genes provides useful information in analyzing unusual isolates.

In addition, identification of electropherotypes provides complementary information on the genomic diversity of rotavirus strains circulating in the region because the migration pattern of 11 segments of double-stranded RNA on a polyacrylamide gel helps define an individual strain of rotavirus (21, 25, 26).

Saudi Arabia is a country characterized by extreme heat and aridity, and it occupies ca. 80% of the Arabian Peninsula. The World Health Organization estimates that there were 462 rotavirus-associated deaths in 2004 (41), and the Ministry of Health reported that there were 267,000 to 439,000 health center visits and approximately 8,000 hospitalizations due to diarrhea of any cause in 2001 (20). However, few studies have addressed the epidemiology of rotavirus in Saudi Arabia. Particularly, information about the distribution of G and P types is completely lacking (20).

The aim of the present study was therefore to determine the distribution of G and P genotypes and electropherotypes of rotaviruses circulating in children less than 5 years of age in Saudi Arabia, in order to examine whether there was any emerging serotype or unusual strain in the region that might challenge the effectiveness of rotavirus vaccines that would be considered for use in Saudi Arabia.

MATERIALS AND METHODS

Detection of rotavirus positive stool specimens by ELISA. Stool specimens were collected from children with acute diarrhea who were referred to the oral rehydration unit (outpatients, $n = 423$) and who were admitted to the hospital (inpatients, $n = 561$) in Maternity and Children's Hospital and Ohod Hospital

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TABLE 1. Oligonucleotide primers used for sequencing of VP7, VP4, and NSP4 genes of MD844

Primer	Gene	Sense	Sequence (5'–3')
End9G12R	VP7	–	TAACGCTAATGAATTTTGG TACTG
AKG12F-01	VP7	+	TTTTCCGCGTTTGCTACC
AKG12F-02	VP7	+	CGCTTCATCTGTTTGTTG
JRG30	NSP4	+	GGCTTTTAAAAGTTCTGTT
JRG31	NSP4	–	ACCATTCCTTCCATTAAC
AKNSF	NSP4	+	GACGTCAGCTAGGATATGA
AKNSR	NSP4	–	CTAGCTGACGTCTCATCTC
AKP8F-01	VP4	+	GCAGTGTTTGAGCTATCG
AKP8F-02	VP4	+	GTAGCCCTCGGTGTTTCA
AKP8R-01	VP4	–	GTAGCCCTCGGTGTTTCA
AKP8R-02	VP4	–	CTATCTACTGGATCGACG

during a 1-year period between 17 April 2004 and 16 April 2005. The two hospitals are the only large general pediatric hospitals providing services for the community of Maddina. The city is one of the largest cities in Saudi Arabia and is situated in the northwest region of the kingdom. A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Rotaclone; Meridian Bioscience, Inc., Cincinnati, OH) was used to detect group A rotavirus antigen.

Extraction of genomic RNA. Rotavirus genomic RNAs were extracted from an ca. 10% suspension of rotavirus-positive specimens in phosphate-buffered saline according to the guanidine isothiocyanate-silica method as previously described (11).

Determination of G and P genotypes. The G types and P types were determined by the method described by Das et al. (8) and Gentsch et al. (11), respectively. Samples that were found G nontypeable were repeated with additional primers, including an alternative G1 typing primer NAC 9 (5), a G5 primer (13) and Beg9 degenerate and End9-degenerate primers to obtain full-length VP7 gene product (14). Samples that were found P nontypeable were repeated with additional primers, including an alternative P[8] typing primer NAC 10 (5) and a degenerate P[8] primer (18). Finally, if the remaining G or P nontypeable samples produced a visible band of an expected size after 30 cycles of reverse transcription-PCR, the product was sent for sequencing.

Culture adaptation. Samples representing different combinations of rotavirus G and P genotypes, as well as major electropherotypes, were chosen for culture adaptation in MA104 cells by using a previously described method (22).

Polyacrylamide gel electrophoresis of rotavirus genomic RNA. Rotavirus genomic RNAs were separated on a 10% polyacrylamide gel by electrophoresis for 18 h at a constant current of 8 mA per gel in a Laemmli buffer system using an SE600 Ruby gel apparatus (GE Health Care Bioscience, Piscataway, NJ) as described previously (21). Electropherotypes were determined by comparison of the individual RNA migration patterns of genome segments on the gel. Short and long RNA patterns were defined by the relative migration of genome segments 10 and 11, with slower-moving genome segments 10 and 11, indicating a short RNA pattern.

Nucleotide sequencing. The double-stranded RNA gene segments 9, 4, and 10 encoding proteins VP7, VP4, and NSP4, respectively, were denatured, reverse transcribed, and then amplified by PCR. For the gene encoding VP7, primers Beg9 and End 9 (14) were used for amplification to obtain a full-length genome segment 9 (Table 1). For the gene encoding VP4, primers con2 and con3 were used to amplify an 877-bp fragment covering the VP8* fragment of genome segment 4 (11). A 739-bp fragment of the NSP4 gene (genome segment 10) was amplified by using primers described previously (7).

Amplicons obtained for each genome segment were purified by using a QIAquick Spin kit (Qiagen, Inc., Valencia, CA). In order to obtain the full sequence in both directions for each genome segment, a series of internal primers was designed (Table 1). Amplicons and primers were sent for sequencing to Lark Technology, Inc., Takeley, Essex, United Kingdom.

Phylogenetic analysis. The nucleotide sequences thus obtained were compared to sequences deposited in the DNA databases by using the BLAST program and the genotypes were determined based on the deduced amino acid sequences that showed the highest identity. Phylogenetic trees were constructed based on the neighbor-joining method (33) in the CLUSTAL W software package (38).

Genogroup analysis. RNA-RNA hybridization was performed as previously described (27). Briefly the ^{32}P-labeled single-stranded RNA probes from reference strains were hybridized to the denatured genomic RNAs from a panel of human rotavirus strains. The human rotavirus strains used in these experiments were Wa (G1P1A[8]), AU-1 (G3 P3[9]), Se585 G12(P2A[6]), I.26 (G12P[4]G12), 04S027 (G12P[8]G12), MD1166 (G2P[6]), MD 713 (G3P[8]), MD409 (G2P[4]), and MD28 (G9P[8]).

Hybridization was allowed to occur at 65°C for 16 h in a buffer containing 25 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, and 0.1% sodium dodecyl sulfate (pH 8.0). The resulting hybrids were then precipitated with ethanol and separated on a 10% polyacrylamide gel. Re-annealed genomic RNAs were visualized by staining with ethidium bromide under UV illumination. Autoradiographs were prepared by exposing dried gels to BioMax MS films (Eastman Kodak Co., Rochester, NY).

Nucleotide accession numbers. The nucleotide sequences for strains MD844 and MD28 have been submitted to the DNA databases, DDBJ, GenBank, and EMBL. The accession numbers of the genes encoding NSP4, VP7, and VP4 of MD844 are AB269688, AB269689, and AB269690, respectively. The accession numbers of the genes encoding VP7 and VP4 of MD28 are AB297791 and AB297792, respectively.

RESULTS

Epidemiologic features of rotavirus diarrhea in Saudi Arabia. Of 984 stool specimens collected from children less than 5 years of age with acute diarrhea between 17 April 2004 and 16 April 2005, rotavirus was detected in 187 (19%) specimens by ELISA. Rotavirus was detected in 158 (28%) of 561 specimens collected from hospitalized children, whereas it was detected in only 29 (7%) of 423 children treated in the oral rehydration unit (outpatients). When the distribution of cases was examined among children less than age 5 years, 159 (83%) occurred in those aged between 4 months and 23 months, whereas only 8 cases (4.3%) occurred in the first 3 months of life (Fig. 1).

Although rotavirus diarrhea occurred throughout the year, there was a seasonal variation in the occurrence of rotavirus diarrhea, with peaks between November and February, where the detection rate of rotavirus-positive cases was 29% on average (Fig. 2). Thus, rotavirus diarrhea was most prevalent in the cooler months of the year in Saudi Arabia.

Molecular characterization of rotavirus genotypes circulating in Saudi Arabia. Of 187 rotavirus-positive specimens, 160 (86%) were successfully classified into G and P genotypes (Table 2): G1P[8] (44%), G2P[4] (20%), G9P[8] (11%), G12P[8] (4%), and G3P[8] (4%). In five specimens (3%) nei-

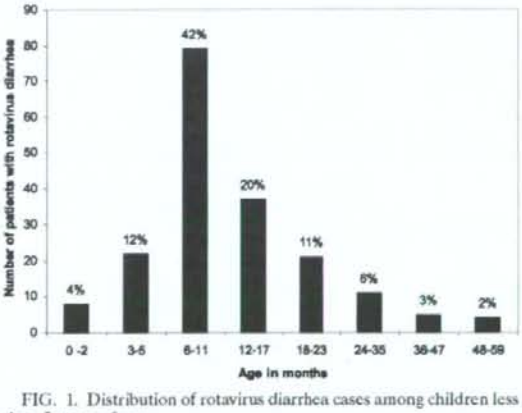

FIG. 1. Distribution of rotavirus diarrhea cases among children less than 5 years of age.

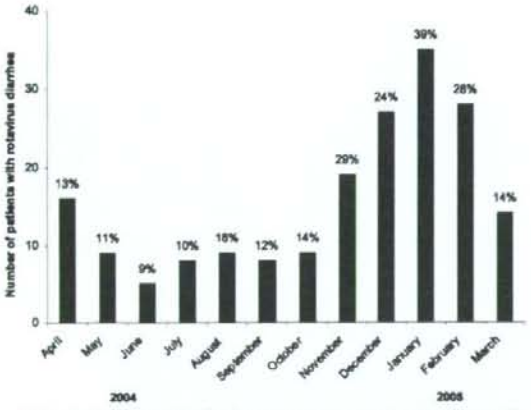

FIG. 2. Temporal distribution of rotavirus diarrheal cases in children less than 5 years of age in Maddina, Saudi Arabia. The percentage shown above each bar indicates the detection rate of rotavirus among all diarrheal cases in the indicated month.

ther the G type nor the P type was detected. In 16 specimens (8%) more than one G or P type was detected, suggesting that they represented a mixed infection of two or more rotavirus strains in a single child. In six specimens (3%) neither G nor P type was determined despite repeated reverse transcription-PCR experiments with different sets of primers, including a G5-specific primer and degenerated primers for genes encoding VP7 or VP4. However, repeated ELISA examination of these specimens confirmed the presence of rotavirus antigen, suggesting the degradation of rotavirus particles in the specimens rather than errors in the initial ELISA testing.

RNA polyacrylamide gel electrophoresis identified 94 (50%) specimens as long RNA patterns, 30 (16%) specimens as short RNA patterns, and one mixed infection, whereas RNA patterns were not assignable in 32 (17%) specimens or were totally undetectable in 30 (16%) specimens (Table 2). As to the relationships between RNA patterns and genotypes (Table 2), all specimens containing short RNA pattern were classified as either G2P[4] (four electropherotypes) or G2P[6] (one electropherotype), whereas all specimens containing long RNA pattern were classified as any one of G1P[8] (four electropherotypes), G3P[8] (two electropherotypes), G4P[8] (1 electropherotype), G9P[8] (two electropherotypes), and G12P[8] (one electropherotype) (data not shown for electropherotypes).

Molecular characterization of the G12 rotavirus detected in Saudi Arabia. Among the specimens that were initially found G nontypeable and P[8], some were isolated in MA104 cells for further characterization. One of these cell-culture-adapted strains, designated MD844, was determined to possess a G12 VP7 gene after sequencing. This prompted us to examine the rest of nontypeable specimens with the G12-specific primer (29, 34), resulting in the identification of six additional G12P[8] specimens, all of which showed an identical long electropherotype (data not shown). The VP7 gene of one of such specimen was sequenced and found to be 100% identical with that of MD844, confirming its G12 specificity (data not shown). Based on these results, it was concluded that there were seven G12 rotavirus specimens of a single strain origin. When the gene encoding VP7 of strain MD844 was analyzed phylogenetically, it was found to belong to the third lineage of G12 VP7 gene, according to Das et al.'s classification (9), which comprise the VP7 genes of G12P[8] and G12P[6] human rotaviruses (Fig. 3).

To further characterize the Saudi Arabian G12 rotavirus, the nucleotide sequences for the genes encoding VP4 and NSP4 of strain MD844 were also determined. The gene encoding VP4 of MD844 was 99% identical to that of a G9P[8] strain, designated MD28, detected and also culture-adapted in the present study, as well as those of many G12P[8] strains reported elsewhere (Table 3). The NSP4 gene of MD844 was determined to belong to genotype B or the Wa-like NSP4 (Fig. 4).

To determine to what extent the G12P[8] isolates were similar in their overall genomic RNA constellation to G12 strains isolated previously elsewhere and to other concurrently circulating genotypes in Saudi Arabia (denoted as strains with the prefix MD), RNA-RNA hybridization was performed with probes made from MD844 (Fig. 5). When the MD844 probe was allowed to hybridize with the genomic RNAs from Se585 (G12P2A[6]), L26 (G12P[4]), newly emerging Nepalese strain 04S027 (G12P[8]) (39), Wa (G1P1A[8]), AU-1 (G1P3[9]), MD1166 (G2P[6]), MD713 (G3P[8]), MD409 (G2P[4]), and MD28 (G9P[8]), the MD844 probe formed seven and nine hybrid bands with the genomic RNAs from MD713 and MD28, respectively. The probe also formed seven hybrid bands with the genomic RNAs from strain 04S027, a Nepalese G12P[8]

TABLE 2. Characterization of rotavirus-positive specimens detected in Maddina, Saudi Arabia, into G and P types and RNA patterns

Parameter	P[8]							P[4]		P[6]			PNT			P[MIX]				Total	%
	G1	G3	G4	G9	G12	GMIX	GNT	G2	GNT	G1	G2	G4	G4	GMIX	GNT	G1	G2	G4	GMIX		
No. of specimens																					
Long	50	5	2	16	7	8	3									1		1	1	94	50
Short						1		27	1		1									30	16
UD[a]	17	1		2				7		1		1	1				2			32	17
Negative	15	1	1	2		1		3						1	6					30	16
Mix								1												1	1
Total	82	7	3	20	7	10	3	38	1	1	1	1	1	1	6	1	2	1	1		
%	44	4	2	11	4	5	2	20	1	1	1	1	1	1	3	1	1	1	1	187	100

[a] UD, undetermined.

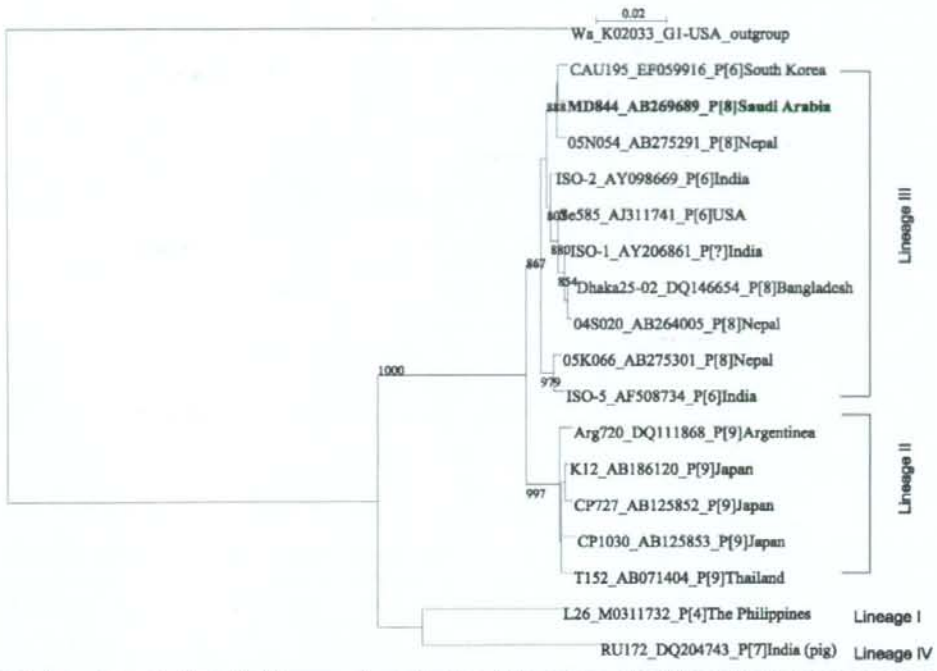

FIG. 3. Phylogenetic tree for the G12 VP7 genes constructed by the neighbor-joining method. The horizontal distance connecting two VP7 sequences is proportional to the genetic distance between these two VP7 sequences. The distance is expressed as the number of the nucleotide substitutions per site. Strain Wa (G1P[8]) was used as an outgroup. The bootstrap probabilities equal or greater than 800 after 1,000 replications are provided at each branching point.

strain. However, the probe formed only one and three hybrid bands with Se585 and L26, respectively. Unexpectedly, the MD844 probe formed only one hybrid band with the genomic RNA of strain Wa, the prototype of the Wa genogroup. The probe formed no hybrid band with AU-1 (the prototype of the AU-1 genogroup), MD1166, or MD409 (both strain most likely to belong to the DS-1 genogroup since they possess a short RNA pattern).

DISCUSSION

The characteristics of rotavirus diarrhea among children less than 5 years of age in Maddina, Saudi Arabia, included a relatively low detection rate (19%) and marked seasonal variation with peaks of infection in the cooler months of the year. However, the rotavirus detection rate obtained in the present study fell well within the expected range since a review of 22

TABLE 3. Comparison of the gene encoding VP4 of Saudi Arabian rotavirus strain MD844 (accession no. AB269690) with other human VP4 genes

Strain	Genotype	Source	Country of origin	% Identity[a]		Accession no.
				nt	aa	
MD28	G9P[8]	Human	Saudi Arabia	99	98	AB297792
B4633-03	G12P[8]	Human	Belgium	98	99	DQ146641
Dahka25-02	G12P[8]	Human	Bangladesh	98	98	DQ146652
Hun9	G9P[8]	Human	Hungary	99	98	AJ605320
DRC88	G8P[8]	Human	Congo	98	99	DQ005111
OP351	G1P[8]	Human	Malawi	99	98	AJ302147
OP601	G1P[8]	Human	Malawi	98	98	AJ302153
CAU202	G9P[8]	Human	South Korea	98	98	EF059923
TF101	G1P[8]	Human	Taiwan	98	97	AF183870
RV161	G12P[6]	Human	Belgium	69	78	DQ490548
Dhaka4-03	G2P[4]	Human	Bangladesh	85	88	DQ482714

[a] Abbreviations: nt, nucleotide; aa, amino acid.

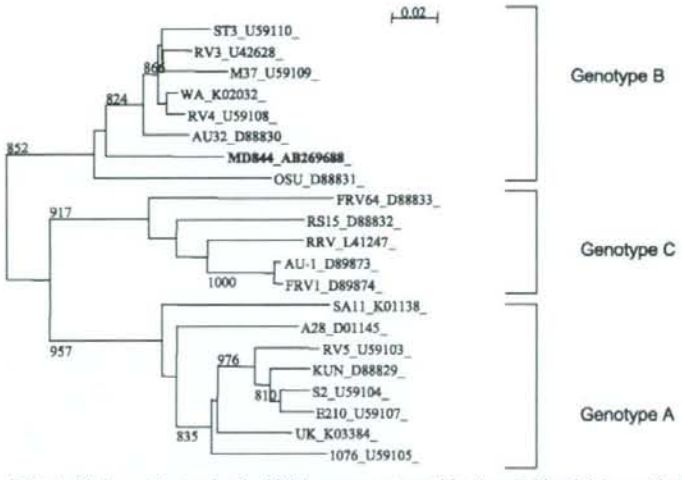

FIG. 4. Phylogenetic tree for the NSP4 genes constructed by the neighbor-joining method.

studies reported in this country showed that the detection rate ranged between 10 and 46% (20). Approximately 60% of rotavirus infections were identified in the first year of life, and 89% were identified by the end of the second year of life. When rotavirus vaccine is introduced into the routine childhood immunization schedule, the first vaccine dose will be given to infants at 2 months of age, meaning that infants younger than this age will not be protected by the vaccine. The observation that only 4% rotavirus infections occurred in the first 3 months of age (Fig. 2) will assure a higher effectiveness of the vaccine in this country according to the proposed immunization schedule.

While this is the first study to describe the distribution of G and P types of rotavirus circulating in Saudi Arabia, there are at least two important observations that merit particular attention. First, we identified here the circulation of G9 strains for the first time in Saudi Arabia at a relative frequency of 11%, confirming and extending the previous observation made in Iran, Iraq, and Kuwait that globally spreading G9 strains have entered the Middle East (1, 10, 24). Although the G9 strains detected in Iran, Iraq, and Kuwait were not sequenced, the VP7 gene that was carried by the G9 strain isolated in Saudi Arabia (MD28) was shown to belong to lineage III (data not shown), a finding consistent with the globally emerging G9 strains (23, 37).

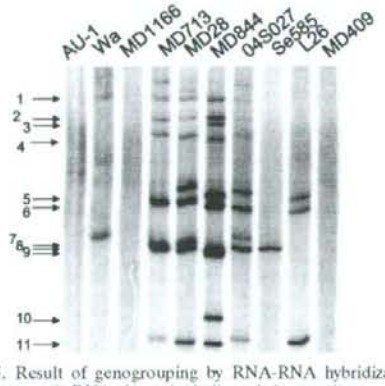

FIG. 5. Result of genogrouping by RNA-RNA hybridization assays. The genomic RNAs from the indicated virus strains were hybridized with the ^{32}P-labeled probe made from MD844. The positions of the RNA segments of the MD844 strain are indicated to the left.

Second, we also identified the circulation of G12 strains for the first time in Saudi Arabia. Although the first detection in the Middle East was in Iran in 2004 (10), there was only one specimen found to contain G12 virus. In this regard it is noteworthy that G12 genotype occurred on multiple occasions, accounting for 4% of rotavirus-positive specimens. Despite the fact the seven specimens containing G12P[8] rotavirus were detected in May 2004 (two specimens), January 2005 (two specimens), and February 2005 (three specimens), there was only a single long RNA electropherotype present in these specimens. This suggests that the introduction of G12 strain into Saudi Arabia was a rather recent event. This observation is in sharp contrast to the observation in Nepal made by Uchida et al. (39) that there were seven electropherotypes for G12P[8] and G12P[6] rotaviruses. Although it is difficult to speculate how such G12P[8] strains were introduced into the country, RNA-RNA hybridization assays provided further information to aid our understanding of the overall genomic RNA relatedness to strains concurrently circulating in the country, as well as to another G12P[8] strain isolated in Nepal. An interesting observation in this context was that strain MD844 was similar in overall genomic constellation to strain MD28, a G9P[8] strain circulating in the region, and, to a lesser degree, to strain 04S027, a G12P[8] strain isolated in Nepal, although no cocirculating G1P[8] strain was included in this comparison. Given that the G12 VP7 gene obtained in the present study showed a high degree of nucleotide sequence

identity with those detected in Nepal and India, it may be that the G12 VP7 gene originating in Asia was introduced into locally cocirculating strains by the mechanism of genetic reassortment. Because G12P[8] strains in Nepal and probably in other South Asian countries have a range of genetic diversity (39), it is also likely that the G12P[8] strain detected in Saudi Arabia could result from the direct introduction of one of the G12P[8] strains circulating in South Asia. In this regard, it may merit mention that there is significant population movement from South Asian countries to Saudi Arabia, which may facilitate the transmission of G12P[8] strains.

Interestingly, G12 strains have now been detected with a variety of gene constellations, including long and short electropherotypes, and in association with three different P genotypes and two different genogroups (2, 3, 9, 30, 31, 35, 36). Such diversity observed in G12 strains strongly suggests the versatility of this strain to spread efficiently in the population through reassortment. Since the study period was limited to 1 year, consideration of the introduction of rotavirus vaccine into Saudi Arabia in the near future requires the need for continuing and broader surveillance of rotavirus strains in circulation.

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Review

Rotavirus genotypes circulating in Brazil before national rotavirus vaccination: A review

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Abstract

Background: Rotavirus vaccine was recently introduced in Brazil, which has the potential to greatly reduce childhood deaths from diarrhoea. To provide baseline data to assess the effect of mass rotavirus vaccination on the ecology of circulating rotavirus strains, we systematically analysed published studies in the pre-vaccine era.

Aims: To describe the distribution of rotavirus genotypes in Brazil prior to vaccine introduction.

Methods: Systematic literature searches in health-related databases from 1986 to 2006. Information extracted and analysed by time and region.

Results: 117 studies with 48,401 participants were included. Of these, 3036 were infected with rotavirus. More than 51 genotype combinations were reported, the distribution of which changed over time.P[8]G1 (43%) was the most frequent genotype throughout, followed by P[8]G9 (22%) and P[4]G2 (7%). The detection rate of P[8]G9 increased, while P[4]G2 decreased during the study period. There was a high frequency of G/P combinations between 1995 and 2000 and a low frequency before and after these years.

Conclusions: While considerable diversity of rotavirus strains was recognized during the pre-vaccine era, three strains comprised 72% of the total analysed. These data provide a baseline against which any changes in circulating rotavirus strains post-vaccine introduction can be measured.

Keywords: Rotavirus; Brazil; Genotypes; Review; Diarrhoea

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1. Introduction

Rotavirus vaccines were recently introduced into national childhood immunization programmes of the USA (Rota Teq®, Merck and Co., Whitehouse Station, NJ, USA), and Brazil, Panama and Venezuela (Rotarix®, Glaxo-SmithKline, Research Triangle Park, NC, USA) (Glass et al., 2006). This represents the most significant public health intervention in the global effort to reduce the morbidity and mortality associated with childhood diarrhoea, since the advent of oral rehydration therapy in the 1950s (Darrow and Pratt, 1950a,b). Rotavirus is the leading cause of severe diarrhoea in infants and young children worldwide. It is estimated to cause 39% of childhood diarrhoea-related hospitalizations. Application of this proportion to the World Health Organization estimates of diarrhoea-related childhood deaths would indicate an estimated 611,000 (range 454,000–705,000) rotavirus-related deaths per year (Parashar et al., 2006). Although its incidence is similar in developed and developing countries (Kosek et al., 2003; Parashar et al., 2003), the case fatality rate among hospitalized children is much higher in low-income countries (Parashar et al., 2003).

Rotavirus, a 70-nm icosahedral virus, is a member of the genus *Rotavirus*, family *Reoviridae* and is a non-enveloped particle with triple-layered wheel-like capsid containing 11 segments of double stranded RNA in its core. Each segment with the exception of the 11th encodes a single protein. Protein VP7 defines the G serotype (for glycoprotein) and VP4 defines the P serotype (for protease-sensitive) (Kapikian et al., 1981). These proteins have independent neutralisation properties and are used in a binary classification system. Based on serological assays, there are 14 P serotypes (nine recovered from humans) and, based on molecular characterization, 26 P genotypes (10 recovered from humans). There are 15 G serotypes, of which 11 (G1–G6, G8–G10, G12 and G15) have been recovered from humans. Because the genes encoding VP7 and VP4 segregate independently, numerous G and P combinations are possible (Hoshino and Kapikian, 2000). The 4 G/P combinations most frequently detected worldwide were reported in 2005 as P[8]G1, P[4]G2, P[8]G3 and P[8]G4 (Gentsch et al., 2005; Santos and Hoshino, 2005), although G9 with both P[6] and mostly P[8] has become one of the most common combination in several places (Kheyami et al., 2008; Rodrigues et al., 2007), including Brazil (Carmona et al., 2006).

The global disease burden attributed to rotavirus infection (Dennehy, 2007; Glass and Parashar, 2006; Glass et al., 2006; Kapikian et al., 1981; Parashar et al., 2003; Santos and Hoshino, 2005) stimulated the development of rotavirus vaccines. The first licensed rotavirus vaccine (RotaShield®, Wyeth Laboratories, Marietta, PA, USA), was a rhesus-human reassortant rotavirus vaccine designed to cover the four most prevalent human G serotypes (G1–G4). Although highly effective in preventing severe rotavirus gastroenteritis, its association with cases of intussusception (Dennehy, 2007; CDC, 1999; Murphy et al., 2001; Simonsen et al., 2001) lead to the withdrawal of the vaccine in 1999, less than 1 year after its introduction (Peter and Myers, 2002; CDC, 1999). Although the strength of this association has been questioned (Simonsen et al., 2005), it nevertheless created additional requirements for larger clinical trials to examine safety issues of two new live, oral, rotavirus vaccines (Ruiz-Palacios et al., 2006; Vesikari et al., 2006).

RotaTeq® is a live, attenuated vaccine comprising a bovine (strain WC3) background (Clark et al., 1996) into which G1–G4 genotypes and the P1A[8] genotype were inserted by reassortment to create a pentavalent vaccine (Heaton et al., 2005). Rotarix® is a live, attenuated vaccine prepared from a G1 P1A[8] human strain isolated from a child (Bernstein et al., 1999). Rotarix® was introduced in the national immunization programme of Brazil in March 2006 with two doses provided free of charge to infants at 2 and 4 months of age, and 1,353,867 vaccine doses were distributed in 2006 (http://www.datasus.gov.br) (Brazil, 2006).

The large-scale introduction of the Rotarix® vaccine in Brazil creates an opportunity to assess its effect on virus ecology, as rotavirus strains for which the vaccine is not fully protective may theoretically emerge through selective immune pressure. If changes in circulating rotavirus strains occur post-vaccine introduction, they will need to be interpreted in the context of their historical prevalence in the country.

This study reviews the frequency and profile changes over time of the rotavirus strains reported from Brazil before vaccine introduction. This information will be useful to assess changes in serotype distribution following the introduction of the rotavirus vaccine, creating a baseline for comparison over time.

2. Methods

A systematic search of the literature was performed in MEDLINE, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (SciELO), Cochrane Library and Pan American Health Organisation (PAHO) Library databases using the keywords "rotavirus" and "Brazil" from 1986 to 2006. Additional searches were conducted using the terms "rotavirus" and "vaccine" in February 2007 to identify papers that quoted subsets of genotypes from Brazil before the introduction of the vaccine. In total, 1330 publications were identified and their abstracts were read. Papers with data on genotype strains, or likely to contain relevant epidemiologic information, were retrieved. One hundred sixty-three papers were identified in MEDLINE (the first database searched); three were identified in LILACS; two in SciELO; and one in the PAHO Library. One hundred and seventeen papers had original data on rotavirus and 58 had rotavirus genotypes. All references cited in these papers were searched to identify further publications, but none was identified. Review or opinion articles without original data or with repeated figures were reviewed and genotype/serotype data was extracted

from the original publications. Pre-formatted tables for the extraction of data were developed and piloted with 10 papers. These included study design, sample size, number of rotavirus positive samples, characteristics of participants (age range, hospitalized/outpatient/community), laboratory methods, region, year, prevalence, genotypes/serotypes identified and frequency. Not all studies reported all of these data and percentages were calculated using as denominator the number of studies reporting each variable.

Information was analysed by time period (before and up to 1995; >1995–2000 and >2000) and region. Brazil is divided into five regions with distinct climate, social and epidemiological patterns. The South (S) and Southeast (SE) regions have humid subtropical and tropical climate, hot rainy seasons from November to January, mild to cold dry winters from May to August and the best social indicators of the country. The North (N) region has Equatorial hot and humid weather all year round, low population density and poor sanitation and health indicators. The West Central (WC) region has tropical climate, rainy and hot summers with large floods, a cold and dry winter and asymmetric health and sanitary conditions. The Northeast (NE) region, the poorest and driest area, has a semi-arid or tropical climate, rainy warm winters and the worst health and sanitary conditions of the country.

3. Results

Over 48,401 individuals participated in the 117 studies included. However, only 6148 of these were tested for rotavirus and 3036 samples were positive. Sixty-three (77%) studies included only children <5 years old and 54 included both children and adults. Fifty-four (60%) studies were cross-sectional, 12 (13.3%) case control studies, 7 (8%) clinical trials and 6 (7%) cohort studies (Table 1). Thirty-eight studies were hospital-based (19 included hospitalized, 20 hospitalized plus ambulatory, and 9 hospitalized plus community-based participants), 25 were community-based, 8 were from ambulatory clinics and 10 did not report the setting (Table 1). There were 3364 (22.4%) positive for rotavirus among 15,033 samples screened. The lowest proportion of positive samples was 3.1% and the highest 40%. Most studies included only <5 years old children (66/81, 82%), seven (9%) included children <10 years old, and eight (10%) included adults. It was not possible to calculate age-specific incidence by region as several publications refer to more than one region.

Twenty-three studies reported both G/P types, 18 only the G, and 10 only the P types. Thirteen studies reported only the presence of antibodies (Andrade et al., 1996; Azeredo et al., 1989; Blake et al., 1993; Candeias et al., 1989; Cardoso et al., 1992; Cox et al., 1998a,b; Gomes et al., 1991; Mehnert and Stewien, 1993; Racz et al., 1988; Santos et al., 1991; Schorling et al., 1990; Timenetsky Mdo et al., 1997). Belém (19 studies), Rio de Janeiro (8) and São Paulo (6) were the cities with the highest number of studies and samples. Table 2 describes the distribution of rotavirus strain types by region and by year. The P[8]G1 strain was reported from all the regions and throughout the study period. Serotype G9 with P[8], P[6] or P[4] VP4 specificity was only reported in the Southeast and West central regions after 1995 and then from all regions after 2000. P[4]G2 was reported throughout the study period from all regions with the exception of the West Central. P[8]G5 strains were reported from all regions before 1995, in the Southeast region from 1995 to 2000, but were not found there after this year. P[6]G4 and P[8]G4 strains were also reported from all regions before 1995, were limited to the Southeast between 1996 and 2000, and spread again to the South, Southeast and West central regions after 2000. P[9]G12 was only reported from the South region after 2000.

A total of 2155 samples reported G types. G1 (947; 44%), G9 (410; 19%) and G2 (355; 17%) were the most frequent, followed by G3 (150; 7%), G4 (96; 5%) and G5 (93; 4%. Others strains included G8, G10 and G12 (total of 9; <1%) and mixed or not typeable (total of 95; 4%) (Fig. 1). Table 3 describes the cumulative frequency and distribution of the genotype combinations over the study periods and Fig. 2 shows how these combinations varied with time and region. The most frequent genotype combinations were P[8]G1 (43%), P[8]G9 (22%), P[4]G2 (7%), P[NT]G1 (5%) and P[8]G4 (4%). There were 51 further P and G combinations representing 20% of the strains including P[6]G2 (3%), P[8]G3 (2%), P[8]G5 (2%), P[8]G2 (2%), P[6]G1, P[4]G3, P[4]G1, P[6]G9, P[4]G9, P[6]G4, P[8]G10, P[9]G3 and others. Mixed infections were reported in 2% of all samples, but the detection rate varied from 2% prior to 1995, 11% between 1996 and 2000, and 1% after 2000. The distribution of strains varied over the study period. P[8]G1 was the most frequent strain type, representing 39% of rotaviruses before 1995,

Table 1
Study design and setting of studies reporting rotavirus genotypes in Brazil, 1986–2006

Study design			Study setting		
	N	%		*N*	%
Cross-sectional	54	60	Hospitalized/outpatients	20	22
Case control	12	13.3	Hospitalized	19	20.9
Cohort	6	6.7	Outpatients	8	8.8
Case report	4	4.4	Community	25	27.5
Bank of specimens	8	8.7	Hospital/community	9	9.9
Clinical trial	7	7.8	Not reported	10	11

Table 2
Genotypes/serotypes reported by region and year, Brazil 1986–2007

Region/year	≤1995	>1995–2000	>2000
North	G1 with P[4], P[6], P[8] G2 with P[4], P[8] G4 with P[6] G5 with P[8], P[6]	G1 with P[8] G2 with P[6]	
Northeast	G1 with P[8] G2 with P[4] G3 with P[8] G4 with P[8] G5 with P[8]		G1 with P[8] G2 with P[4] G8 with P[6] G9 with P[8]
Southeast	G1 with P[1], P[2], P[3], P[6], P[8] G2 with P[2], P[4] G3 with P[1], P[3], P[6], P[8] G4 with P[1], P[8] G5 with P[8]	G1 with P[8], P[9], P[6] G2 with P[4], P[6], P[8] G3 with P[4], P[6], P[8], P[9] G4 with P[6], P[8] G5 with P[8] G9 with P[4], P[8]	G1 with P[4], P[6], P[8] G2 with P[4], P[8] G3 with P[8] G4 with P[8], P[9] G8 with P[4] G9 with P[4], P[6] G10 with P[4]
West Central	G1 with P[8] G3 with P[8] G4 with P[8] G5 with P[8]	G10 with P[8] G1 with P[8] G1 with P[6] G2 with P[6], P[8] G9 with P[6], P[8]	G1 with P[6], P[8] G4 with P[8] G9 with P[8]
South	G1 with P[8] G2 with P[4] G4 with P[8] G5 with P[8]		G1 with P[8] G9 with P[8] G12 with P[9]

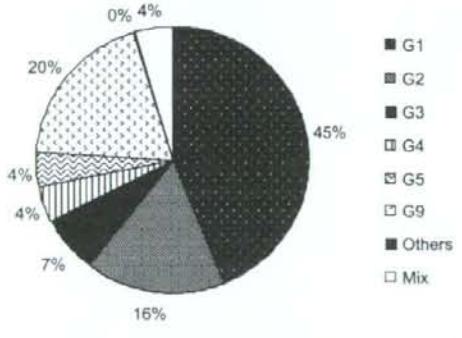

Fig. 1. G Rotavirus genotypes/serotypes in Brazil, 1986–2006.

10% from 1996 to 2000, and 54% after 2000. P[8]G9 strains increased from 0% before 1995 to 8% and 31% between 1996–2000 and after 2000, respectively. P[4]G2 decreased from 19% before 1995 to 12% and 1% in the subsequent periods, respectively. The cumulative proportion of cases with P[8]G1, P[8]G9 and P[4]G2 varied from 58% before 1995 to 30% and 86% in 1996–2000 and after 2000, respectively. One hundred and seventy-nine (75%) of the specimens reported in studies published before 1995, 195 (54%) reported between 1996 and 2000, and 919 (96%) of specimens reported after 2000 shared at least one of the G or P types present in the Rotarix® vaccine. Two hundred and thirty-five (98%) specimens reported before 1995, 321 (89%) between 1996 and 2000, and 935 (97%) after 2000 shared at least one of the G or P types of the RotaTeq® vaccine.

4. Discussion

Brazil participated in clinical trials for the evaluation of the Rotarix® vaccine (Linhares et al., 2006; Ruiz-Palacios et al., 2006; Salinas et al., 2005) and decided to pioneer this vaccine in Latin America together with Mexico, Venezuela and Panama, creating an unprecedented situation for the control of childhood diarrhoea (Dennehy, 2007). Although the trials generated a large amount of information on the rotavirus genotypes circulating at the time of the studies, there was no systematic review of the genotypes that had circulated in Brazil in previous years. A global review of the distribution of rotavirus serotypes (Santos and Hoshino, 2005) included 16 publications from Brazil and concluded that P[8]G1 was the most frequent strain, but that some uncommon strains such as G5, and G9 were emerging. These emerging strains were subsequently reported from Rio de Janeiro (Santos et al., 2001), Sao Paulo (Carmona et al., 2006), Salvador (Santos et al., 2005), and Recife (Montenegro et al., 2007). As G and P genotypes are known to vary over time and region, these short-term changes may provide an incomplete understanding of the most frequent genotypes occurring over a longer period.

The distribution of genotypes in Brazil in the time period reviewed was similar to their global distribution, although

Table 3
Cumulative G and P combinations by study period, Brazil 1986–2006[a]

G/P combination	≤1995		>1995–2000		>2000		Total	
	N	%	N	%	N	%	N	%
P[8]G1	111	46	40	11	524	54	675	43
P[8]G9	0	0	30	8	307	32	337	22
P[4]G2	56	23	47	13	9	1	112	7
P[NT]G1	9	4	47	13	17	2	73	5
P[8]G4	9	4	8	2	50	5	67	4
P[6]G2	0	0	42	12	1	0	43	3
P[8]G3	7	3	22	6	3	0	32	2
P[8]G5	16	7	8	2	6	1	30	2
P[8]G2	7	3	21	6	0	0	28	2
P[6]G1	7	3	11	3	3	0	21	1
P[4]G3	0	0	18	5	2	0	20	1
P[4]G1	8	3	0	0	9	1	17	1
P[NT]G9	0	0	7	2	9	1	16	1
P[NT]G2	1	0	8	2	4	0	13	1
P[6]G9	0	0	9	3	1	0	10	1
P[4]G9	0	0	4	1	5	1	9	1
P[6]G4	4	2	5	1	0	0	9	1
P[8]G10	0	0	8	2	0	0	8	0
P[9]G3	0	0	6	2	0	0	6	0
Mixed/others	5	2	19	5	11	1	35	2
Total	240	100	360	100	961	100	1561	100

[a] A full list of references can be obtained from the corresponding author.

with a prevalence that varied over time. P[8]G1 was the most frequent strain type reported before 1995 and after 2000, while P[4]G2 decreased from 19% before 1995 to 1% after 2000. P[8]G9 and P[9]G9 were not reported before 1995 but had become the second most frequent strains after 2000 (Araujo et al., 2007; Carmona et al., 2006; Montenegro et al., 2007; Santos et al., 2005). The increase in these latter genotypes were also reported from other South American (Parra et al., 2007), European (Rodrigues et al., 2007) and Asian countries (Khamrin et al., 2006), but its prevalence seems to be decreasing in the most recent reports (Khamrin et al., 2007). The pattern of G and P combinations in Brazil were not characteristic of the patterns observed either in developing or industrialised countries. In the former, large numbers of genotypes are often reported simultaneously (Castello et al., 2004; Iturriza-Gomara et al., 2000; Kang et al., 2005; Koshimura et al., 2000), while in the latter the number of strain types is usually relatively small (Santos and Hoshino, 2005). Before 1996, two genotypes (P[8]G1 and P[4]G2) represented 69% of characterized strains, between 1995 and 2000 there was a large number of G/P combinations and after 2000, two genotypes (P[8]G1 and P[8]G9) represented 75% of the strains. Although the large variety reported in the intermediate period (1995–2000) may be the result of positive reporting bias during the time leading to the introduction of the vaccines, the different genotypes predominating over time illustrate that substantial changes have occurred and that these changes are not necessarily homogeneous across the regions. A previous review of the global distribution of rotavirus genotypes (Gentsch et al., 2005) reported the wide variety of strains encountered in Brazil, and described 80 rare strains in Brazil, while in the remaining studies con-

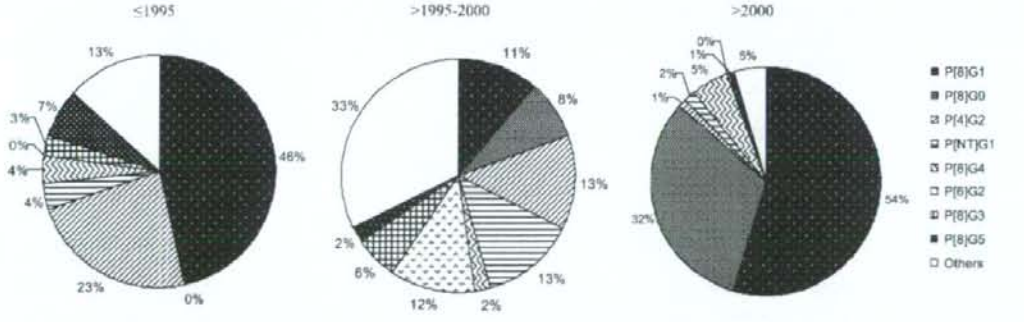

Fig. 2. Rotavirus G and P genotype/serotype combinations by study period.

ducted elsewhere, there were 314 strains considered to be rare.

Rotavirus evolution in nature is a result of genetic reassortment, accumulation of point mutations including in the genes encoding VP4 and VP7, and interspecies rotavirus transmission (Iturriza-Gomara et al., 2001; Laird et al., 2003; Santos et al., 2003; Unicomb et al., 1999). In Brazil, as elsewhere, the emergence of serotype G9, initially with P[6], but more frequently with P[8] may be have occurred through reassortment (Araujo et al., 2001; Oka et al., 2000; Ramachandran et al., 2000; Santos et al., 2001). Strain P[8]G5, frequently detected (7%) before 1995, but rarely detected (2% and 1%) in the two most recent periods, may have been introduced by interspecies transmission from a porcine origin (Gouvea et al., 1994a,b). A later report of serotype G10 probably derived from a bovine (B223) strain (Volotao et al., 2006) and the detection of serotype G12 (Pietruchinski et al., 2006), which is closely related to an Argentinean strain (Castello et al., 2006) and followed the widespread emergence of this genotype in USA (Griffin et al., 2002), Thailand (Pongsuwanna et al., 2002), India (Samajdar et al., 2006) and Japan (Shinozaki et al., 2004), reinforces the large diversity of the Brazilian genotypes and the multiple ways that rotaviruses may evolve in this environment.

Rotarix® has 84.7% overall efficacy against severe rotavirus diarrhoea, and 85% against hospitalization by rotavirus diarrhoea. The efficacy against severe episodes caused by P[8]G1 strain, homologous to the vaccine, was 90.8% and the vaccine appears protective against non-G1 strains that share the P[8] genotype (e.g. 87.3% for the P[8]G9 combination) (Ruiz-Palacios et al., 2006). However, if other genotypes such as G6 or G12 emerged (Bernstein and Ward, 2006; Grimwood and Buttery, 2007; Santos and Hoshino, 2005), vaccine efficacy may be more challenge, especially if such strains do not possess the P[8] VP4 genotype. Although the mechanisms of protective immunity against rotavirus infection are incompletely understood, the possibility that vaccine efficacy may vary by strain type was anticipated before vaccine introduction (Gentsch et al., 2005; Perez-Vargas et al., 2006).

As a large proportion of the rotavirus strains reported in the 5 years before the introduction of the vaccine shared at least one of the G and P proteins with the current vaccines (83% for Rotarix® and 96% for RotaTeq®), vaccine efficacy would not be challenged by heterotypic strains. Recent reports from northeast Brazil, after the introduction of Rotarix®, indicated that all strains identified in 2007 belong to the P[4]G2 genotype (Gurgel et al., 2007; Nakagomi et al., 2008), raising the possibility that emergence of strains that do not share either of the vaccine's two surface proteins could occur. However, these observations could have occurred by chance, and therefore continued surveillance is required to confirm or refute this hypothesis.

Brazil was the first country to introduce one of the two current rotavirus vaccines into its national immunization program and has therefore the largest and oldest vaccinated birth cohort in Latin America. The Rotarix® vaccine is provided free of charge for all children and has attained a high coverage. The potential to reduce the burden of diarrhea and improve the lives of millions of children is enormous. However, post-marketing surveillance and a good understanding of the historical genotype profiles circulating in the decades before the introduction of the vaccine, as provided here, are crucial to enable proper interpretation of the broader impact of the vaccine in the future.

Conflicts of interest

RQG and LEC have no conflicts of interest to declare. NAC has received funds for rotavirus research from GSK Biologicals and SPMSD. ON has received funds for rotavirus research from GSK, Japan.

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