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A hospital based study on inter- and intragenotypic diversity of human rotavirus A VP4 and VP7 gene segments, Germany

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ABSTRACT

Background: Efforts to reduce the impact of group A rotaviruses on human morbidity and mortality rely on oral immunisation with live attenuated or recombinant vaccines. A major challenge in immunisation is the vast inter- and intragenotypic diversity accomplished by circulating rotaviruses.
Objectives: To monitor rotavirus inter- and intragenotypic diversity in hospitalised children.
Study design: From January 2008 to December 2009 stool samples from 1994 paediatric in-patients suffering from diarrhoea were screened for rotavirus. Rotavirus G- and P-genotypes were determined by nucleotide sequencing and phylogenetic analysis was performed.
Results: Rotavirus A was detected in stool samples of 341 children, comprising G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], as well as uncommon G12P[6] genotypes and mixed infections. Predominant strains shifted from G1P[8] and G9P[8] genotypes in the first season to G3P[8] and G4P[8] genotypes in the second season. The highest intragenotypic diversity was detected in G1 strains and consisted of co-circulating G1-Ic, G1-Id, G1-Ie and G1-II rotaviruses. The G2 analysis revealed different intragenotypic lineages: G2-IIa, G2-IIb and G2-IIc. Interestingly, the circulating G4-Ib rotaviruses were characterised by insertions of 3 or 6 additional coding nucleotides within variable region 4 of VP7. Whereas different G9-III VP7 gene segments were detected G3-Ia sequences were highly homologous. In the VP4 analysis P[8]-III gene segment predominated over P[4]-Vb, P[8]-I, P[8]-IV and P[6]-I.
Conclusions: A remarkable rotavirus heterogeneity was detected in the limited local setting and time span. Continued monitoring and nucleotide sequencing is necessary to document possible effects of rising immunisation levels on intragenotypic rotavirus diversity.

1. Background

Rotaviruses are enteric pathogens in humans and animals. They comprise a genus in the *Reoviridae* family and are classified into seven groups (A–G) based on their distinct antigenic and genetic properties.[1] Group A rotaviruses are the leading cause of severe gastroenteritis in infants and accountable for more than 600,000 lethal cases in children per year.[2] Efforts are being made to reduce the mortality of rotavirus by vaccination.[3,4]

A major challenge in immunisation is the vast inter- and intragenotypic diversity accomplished by group A rotaviruses. The variety in the rotaviral genome is a consequence of several mechanisms: reassortment, rearrangement and point mutations.[5] In order to cover the frequent occurrence of reassortment events a separate genotype is assigned to each of the 11 rotaviral dsRNA segments.[6,7] Immunologically most important are the VP4 and the VP7 gene segments, which code for the outer layer capsid proteins, which elicit the production of neutralising antibodies.[1] G and P genotypes are assigned according to partial VP7 and VP4 sequences, respectively.[6] The most prevalent G and P genotype combinations in humans are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].[2] So far more than 27 P and 19 G genotypes have been described and are subdivided into several intragenotypic lineages.[6,7]

Two oral live vaccines are in use in Germany since 2006: Rotarix consists of the attenuated G1P[8] human rotavirus strain RIX4414.[3] RotaTeq comprises five bovine-human recombinant rotaviruses, representing human genotypes G1 to G4 and P[8].[4]

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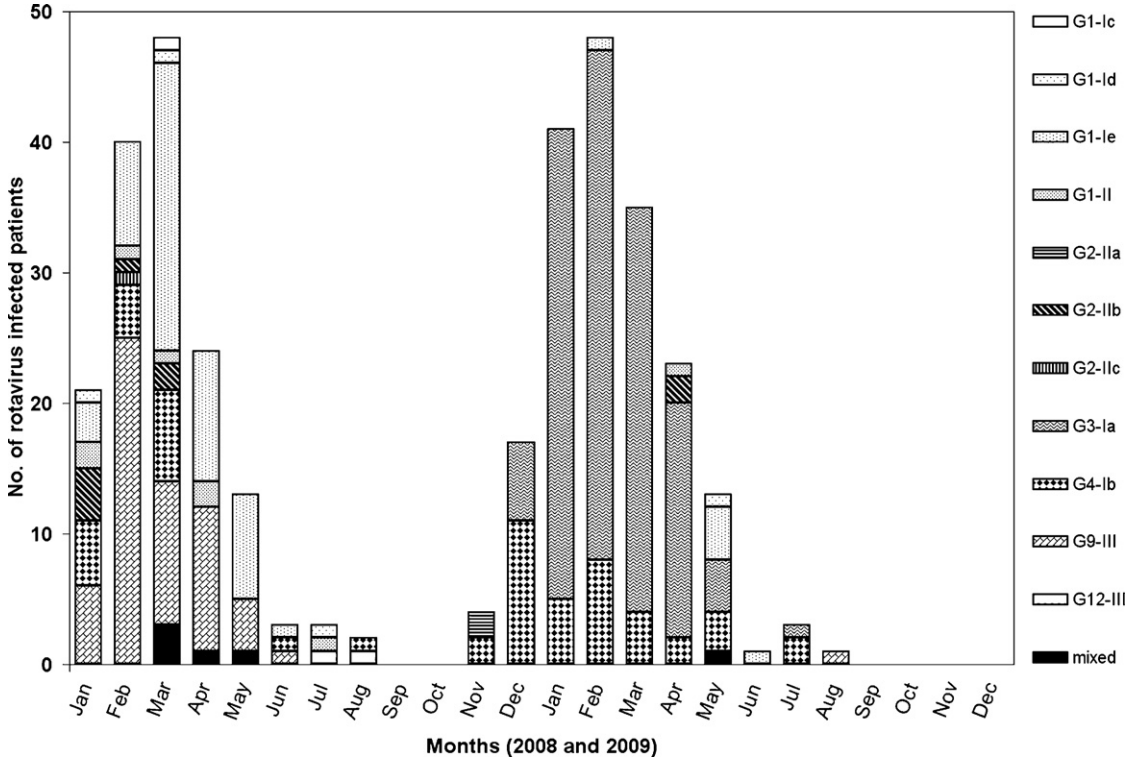

Fig. 1. Prevalence of G genotypes from January 2008 to December 2009. The number of infected patients per months is given and is itemised up to the different intragenotypic lineages.

The total rotavirus immunisation coverage in the Leipzig region is estimated as 36.2% and 56.8% of infants in 2008 and 2009, respectively (Dr. D. Beier, Landesuntersuchungsanstalt Sachsen, personal communication).

2. Objectives

To monitor rotavirus inter- and intragenotypic diversity in hospitalised children in a major city in central Germany.

3. Study design

From January 2008 to December 2009 stool samples of 1994 hospitalised children (under the age of 18 years) at Leipzig University Hospital, were screened for presence of rotavirus (Rotavirus IDEIA, Dako Ltd., Cambridgeshire, UK). RNA was extracted by NucliSens easyMAG system (bioMérieux, Boxtel, The Netherlands) and rotavirus infection was confirmed by real-time PCR.[8] The VP7 gene segment and a section of VP4, coding for the tryptic cleavage product VP8, were amplified using the modified primer Beg9 (5′-CTTTAAAAGAGAGAATTTCCGTCTGG-3′) and primer End9, and primer con2 and the modified primer con3 (5′-GCTTCGCCATTTTATAGACA-3′), respectively.[9,10]

Gel purified amplicons (Wizard SV Gel and PCR Clean-Up System; Promega, Mannheim, Germany) were partially sequenced (approximately 600 bp each) using BigDye Terminator v1.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA) on an ABI Prism 310 Genetic Analyzer. Nucleotide sequences read from the chromatograms were aligned. Sequencing of the entire amplicons was done for selected rotavirus isolates: a VP8 or VP7 amplicon was entirely sequenced, if either VP8 or VP7 partial nucleotide sequences showed below 99% identity to an already characterised strain of the study.

In the phylogenetic analysis the complete coding nucleotide sequences of VP7 and nucleotide sequences of codon 9–240 of VP8 of the representative strains were aligned to published sequences from GenBank and phylogenetically analysed using MEGA, version 4.0, software.[11] Genetic distances were calculated using the Poisson correction parameter. The dendrograms were constructed by the neighbor-joining method. Statistical support was assessed by bootstrapping with 1000 replicates.

4. Results

From January 2008 to December 2009 stool samples of 341 (17.1%) children were reactive in ELISA. All were confirmed by PCR and subsequently G and P genotyped. The age of the 189 males and 152 females rotavirus infected patients was between

Table 1
Number of rotavirus strains of the respective G and P genotype combinations in two consecutive seasons monitored between January 2008 and December 2009.

	P[4]-Vb	P[6]-I	P[8]-I	P[8]-III	P[8]-IV	total
G1-Ic				1/0		1/0
G1-Id				2/1	1/0	3/1
G1-Ie				52/6		52/6
G1-II			6/0	1/1		7/1
G2-IIa	0/2					0/2
G2-IIb	8/2					8/2
G2-IIc	1/0					1/0
G3-Ia				0/136		0/136
G4-Ib				18/36		18/36
G9-III				58/1		58/1
G12-III		2/0				2/0
G1-Ie+G3-I				0/1		0/1
G1-Ie+G9-III				4/0		4/0
G3-Ia+G12-III				1/0		1/0
Total	9/4	2/0	6/0	137/182	1/0	155/186