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Rotavirus Diversity and Evolution in the Post-Vaccine World

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Abstract

Rotaviruses (RVs) are a large genetically diverse population of segmented double-stranded (ds) RNA viruses that are important causes of gastroenteritis in many animal species. The human RVs are responsible for the deaths of nearly 450,000 infants and young children each year, most occurring in developing countries. Recent large-scale sequencing efforts have revealed that the genomes of human RVs typically consist of phylogenetically linked constellations of eleven dsRNA segments. The presence of such preferred constellations indicate that the human RV genes have co-evolved to produce protein sets that work optimally together to support virus replication. Two of the viral genes encode virion outer capsid proteins (VP7 and VP4) whose antigenic properties define the G/P type of the virus. From year-to-year and place-to-place, the G/P type of human RVs associated with disease can fluctuate dramatically, phenomena that can be associated with the presence and behavior of genetically distinct RV clades. The recent introduction of two live attenuated RV vaccines (RotaReq™ and Rotarix™) into the childhood vaccination programs of various countries has been highly effective in reducing the incidence of RV diarrheal disease. Whether the widespread use of these vaccines will introduce selective pressures on human RVs, triggering genetic and antigenic changes that undermine the effectiveness of vaccinations programs, is uncertain and will require continued surveillance of human RVs.

Keywords

rotavirus; genome; genomics; vaccine

Introduction

Rotaviruses (RVs) were first described in 1973 (Bishop *et al.*, 1973). Thereafter, they were quickly recognized as important causes of diarrheal disease in many species of animals, including humans (Estes & Kapikian, 2007). Today, we know that the group A RVs are responsible for ~1500 deaths each day, primarily of infants and young children in developing countries (Yen *et al.*, 2011b). We have also learned that there are numerous distinct types of RVs; some are found throughout the world while others seem to remain regional, and yet others can be seen to emerge, then disappear, only to re-emerge later (O'Ryan, 2009; WHO, 2010). While much remains to be learned about the human RVs, much has changed recently. Most notably, effective RV vaccines have been introduced in many countries of the world (Heaton & Ciarlet, 2007; Ruiz-Palacios *et al.*, 2006). In addition, global RV surveillance programs have been established (Iturriza-Gomara *et al.*, 2011; Payne *et al.*, 2008; Payne *et al.*, 2011; Steele & Ivanoff, 2003; Steele *et al.*, 2003), high throughput sequence technologies have allowed large scale complete genome sequence projects to move forward (McDonald *et al.*, 2009), and sophisticated classification schemes

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and phylogenetic analyses (Matthijnssens *et al.*, 2008a) are providing new insight into the dynamic landscape of circulating RVs. As reviewed below, information gained from these changes has advanced our understanding of RV biology and epidemiology, while bringing some clarity to the challenges that are faced in developing a globally successful immunization program.

Rotavirus Structure

The infectious RV virion is a 100-nm non-enveloped icosahedral particle with a capsid made up of three concentric protein layers (Fig. 1A-C) (McClain *et al.*, 2010; Settembre *et al.*, 2011). The capsid surrounds a genome composed of eleven segments of double-stranded (ds) RNA (Fig. 1D). Each genome segment codes for one protein, except for segment 11, which can code for two (Estes & Kapikian, 2007). Six of the proteins are structural components of the capsid (VP1-VP4 and VP6-VP7) and six are nonstructural proteins (NSP1-NSP6) (Fig. 1D). The nonstructural proteins support various virus functions, including genome replication, particle assembly, regulation of host innate responses (e.g., interferon induction) (Feng *et al.*, 2008), and stimulation of viral gene expression. The innermost protein layer of the virion is formed by the core shell protein VP2 (McClain *et al.*, 2010). Attached to the interior side of the VP2 layer are two minor proteins: the viral RNA-dependent RNA polymerase VP1 and the RNA capping enzyme VP3 (Liu *et al.*, 1992; Lu *et al.*, 2008). Together, VP1, VP2, VP3 and the dsRNA genome form the virion core. The core is surrounded by VP6, the sole component of the intermediate protein layer (Fig. 1B). The outer protein layer of the virion consists mostly of the VP7 glycoprotein. Projecting outward from the VP7 layer are spikes of the protease-activated attachment protein VP4. Cleavage of the VP4 spike by trypsin-like proteases yields two polypeptides, VP8* and VP5* (Arias *et al.*, 1996). VP8* represents the globular head of the spike, while VP5* forms its stalk and base (Fig.1D) (Settembre *et al.*, 2011). In the infected host, intestinal trypsin-like proteases cleave VP4, stimulating virus interaction and penetration of susceptible intestinal enterocytes.

The RV VP7 and VP4 proteins (including the VP4 fragments VP8* and VP5*) contain multiple antigenic epitopes that can induce the production of neutralizing antibodies (Aoki *et al.*, 2009; Dormitzer *et al.*, 2002). With the availability of atomic structures for VP7 and VP4 and the characterization of viral neutralization escape mutants, it has been possible to locate and identify amino acids critical to the antigenic properties of RVs. This information combined with sequencing of RV VP7 and VP4 genes provides a pathway for monitoring circulating human viruses for antigenic changes that may influence the effectiveness of RV vaccines.

Classification and Diversity

Groups

RVs (genus *Rotavirus*) are members of the *Reoviridae*, a family of icosahedral viruses with genomes consisting of 9 to 12 segments of dsRNA (Attoui *et al.*, 2012). RVs are subdivided into groups (or species) based on the antigenic properties or, more recently, the amino acid sequences of the VP6 capsid protein (Matthijnssens *et al.*, 2012). So far, five groups (A to E) have been defined for RVs, with phylogenetic evidence suggesting the existence of three more (groups F to H). Virus strains belonging to different RV groups appear not to be able to exchange genome segments, i.e., reassort their genomes, during co-infection. In contrast, virus strains belonging to the same group can reassort their genomes, providing a mechanism for the evolution of novel viruses (Muller & Johne, 2007).

Of the various RV groups, viruses belonging to group A account for nearly all RV-associated mortality and morbidity. It is these viruses that are targets of current vaccine programs. A number of group B, C, and H RVs have been identified that can cause diarrheal disease in humans (Attoui *et al.*, 2012). Perhaps most notable of these is the group B adult diarrhea rotavirus (ADRV) (Fang *et al.*, 1989). Unlike the group A RVs, which are associated with disease predominantly in children less than 5 years of age, ADRV infections have caused large outbreaks of severe diarrhea involving thousands of adults in China. Group C RVs are associated with diarrheal disease in children that are somewhat older (4 to 7 years) than is typical of group A infections (Caul *et al.*, 1990; Matsumoto *et al.*, 1989). Group C outbreaks tend to be sporadic and self-limiting in nature, and have been associated with food-borne contamination in institutional settings. The group D, E, and G RVs are only known to infect avian species (Trojnar *et al.*, 2010).

G and P types

In early studies of group A RVs, virus strains were assigned G and P serotypes simply based on the reactivity of the antigenic epitopes of their VP7 (G for Glycoprotein) and VP4 (P for Protease-sensitive) proteins to reference antisera (Coulson, 1996). More recently, the binary G/P-serotyping system of classifying RV strains has been largely replaced with a G/P-genotyping system that is based on analysis of VP7 and VP4 genes by reverse transcription-polymerase chain reaction (i.e., RT-PCR typing) or by cDNA sequencing (Gentsch *et al.*, 1992; Gouvea *et al.*, 1990). Currently, 27 G genotypes (G1 - G27) and 35 P genotypes (P[1] - P[35]) have been described for RVs (Matthijnssens *et al.*, 2011a). To what extent each genotype defines an antigenically distinct VP7 or VP4 protein is not known.

Globally, the vast majority of human RVs associated with diarrheal disease have the genotype combinations G1P[8], G2P[4], G3P[8], G4P[8], or G9P[8] (Santos & Hoshino, 2005; WHO, 2010). In developed countries, these (common global) strains may cause nearly 100% of infections in some RV seasons (see Fig. 2) (Iturriza-Gomara *et al.*, 2011; Iturriza-Gomara & Gray, 2011; Payne *et al.*, 2011). Many, if not all, of the common human strains may co-circulate within a single season, creating conditions that favor the formation of reassortant viruses (Payne *et al.*, 2009; WHO, 2010). Of the common global strains, the G1P[8] viruses consistently represent *on average* the primary cause of human disease. However, at any one location, strains that are other than G1P[8] may be primarily responsible for disease, and from one season to the next, the genotype of the viruses primarily responsible for disease may change (O'Ryan, 2009; Payne *et al.*, 2009; Zuridah *et al.*, 2010). The epidemiological basis of the genotype cycling phenomenon is unclear, but it does introduce challenges to predicting the appropriate composition and the efficacy of vaccines.

In developing countries, human RV strains with uncommon G/P type combinations, due to reassortment with animal RVs, can be a frequent cause of disease in young children (Fig. 3) (Armah *et al.*, 2010; Binka *et al.*, 2011; Jere *et al.*, 2011b). Remarkably, the G/P types of the uncommon strains show wide variation from one region to the next. For instance, a surveillance program directed by the World Health Organization noted that in 2010 the predominant uncommon strains were G12P[8] and G12P[6] viruses in Southeast Asia; G2P[6], G3P[6], and G1P[6] viruses in sub-Saharan Africa; G1P[4] and G2P[8] viruses in the Western Pacific; and G9P[4] viruses in the Americas (WHO, 2010). Which, if any, of the uncommon strains will spread throughout the world to become common global strain is difficult to predict. Indeed, the G9P[8] strains represent the only clear example of a previously rare G/P genotype combination that has become dominant within the landscape of globally circulating RVs (Clark *et al.*, 2004; Cunliffe *et al.*, 2001; Matthijnssens *et al.*, 2010a). Based on the increasing numbers of countries, both developed and developing, that have reported human G12 RV infections during the last 10 years, it is possible that G12

viruses will become globally dominant as well (Matthijnssens et al., 2009; Rahman *et al.*, 2007; Samajdar *et al.*, 2006).

Gene constellations

The limitation of the binary G/P type classification system is that it ignores all but two (VP7 and VP4) of the eleven viral genes. Thus, the binary system fails to provide the necessary information required to fully evaluate the genetic diversity and evolutionary dynamics and relationships of co-circulating RVs. This limitation has been partially overcome by two developments: (i) the advancement of high throughput sequencing technologies that allow routine full-genome sequencing of RV strains (e.g., http://gsc.jcvi.org/projects/msc/rotavirus/) and (ii) the creation of a complete sequence-based classification system that allows each genome segment of the virus to be assigned to a particular genotype (Matthijnssens *et al.*, 2008a). In this classification system, the genome segments for VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 are represented by the acryonym Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (x = Arabic numerals > 1). The VP7 and VP4 genotypes used in the full genome classification system are the same as described above for the binary G- and P-typing system. To date, 8 or more genotypes have been defined for each of the other nine segments (termed the internal genes) of RV strains (Table 1).

Full-genome sequencing has revealed that the internal genes of human G1P[8], G3P[8], G4P[8], and G9P[8] RVs almost invariably belong to genotype 1 (Table 1) (Heiman *et al.*, 2008; Jere *et al.*, 2011a; Matthijnssens *et al.*, 2008a; McDonald *et al.*, 2009). When there are exceptions, usually only 1 or 2 of the internal genes are involved (Esona *et al.*, 2011). Viruses that have internal gene constellations that are predominantly of genotype 1 are referred to as genogroup 1 viruses. Sequencing has revealed that the internal genes of the human G2P[4] viruses typically belong to genotype 2. Viruses with such internal gene constellations are referred to as genogroup 2 viruses (Table 1). Genome sequencing studies indicate that RV strains with pure genotype 1 or 2 internal gene constellations are rarely recovered from animals, other than humans. This suggests that viruses with pure genotype 1 or 2 constellations are ideally suited for replication in humans. Phylogenetic analysis has indicated that a distant evolutionary link exists between human genogroup 1 RV and porcine RVs and between human genogroup 2 RVs and bovine RVs (Matthijnssens *et al.*, 2008a). This evolutionary link accounts for the many genotype 1 genes that the human G1P[8], G3P[8], G4P[8], and G9P[8] viruses share with some porcine RVs (e.g., OSU) and for the many genotype 2 genes that the human G2P[4] viruses share with some bovine RVs (e.g., WC3) (Table 1).

Rotavirus Disease

Group A RVs are the primary cause of acute dehydrating diarrhea in infants and children under 5 years of age (Bernstein, 2009). These viruses are transmitted by the fecal-oral route, and peak periods of RV-associated disease occur within the winter-spring months in temperate climates (Cook *et al.*, 1990). Significant numbers of children can have asymptomatic infections, yet shed virus in their stool, thus serving as possible sources of virus within the community (Ramani *et al.*, 2010). Similarly, RVs can cause symptomatic and asymptomatic infections in older children and adults; a common feature that may contribute to the rapid global spread of the virus (Anderson & Weber, 2004).

In addition to humans, RVs are responsible for gastroenteritis in many other animal species, including common farm animals (cows, pigs, sheep), exotic animals (llamas, giraffes), nonhuman primates (macaques), house-hold pets (dogs, cats), rodents, and birds (Martella *et al.*, 2010). The gene constellations of animal viruses are often unique and quite different in

their genotype composition from that of human RVs, an attribute that likely reflects the co-evolution (or co-speciation) of animal viruses with their natural host (Matthijnssens *et al.*, 2011c). Perhaps, it is this co-speciation process that explains why it is rare for zoonotic infections to lead to large-scale outbreaks of human disease (Martella *et al.*, 2010). Even when administered at high titers, animal RVs usually fail to cause diarrheal disease in humans (Vesikari *et al.*, 1986). However, animal RVs can induce protective immunological responses against RV disease when administered to humans, which has provided the conceptual basis for the development of some RV vaccines (Christy *et al.*, 1988; Vesikari, 1996).

In essence, the gene products of animal viruses may not function well enough in humans to support efficient virus replication and spread. However, there are numerous reports in the literature of animal-like RVs causing disease in humans (Ghosh & Kobayashi, 2011; Matthijnssens *et al.*, 2011b; Matthijnssens *et al.*, 2008b; Park *et al.*, 2011). In many cases, these animal-like viruses appear to have originated by reassortment between an animal and human RV, generating novel virus strains with gene constellations that include one or more genes that are typical of human viruses. The acquisition of human RV genes may improve the efficiency of the virus such that it can productively replicate in humans, leading to disease outbreaks.

The major symptoms associated with RV disease in young children are mild-to-severe watery diarrhea and vomiting (potentially leading to dehydration), and low-grade fever, with symptoms lasting for up to 4–8 days (Bernstein, 2009). The primary site of replication is the mature enterocytes at the villus tips of the small intestine (Greenberg & Estes, 2009). Infection characteristically leads to blunting of the villi and defects in fluid absorption and retention and ion transport. Recent studies have revealed that RV infections may spread outside the intestine, leading to antigenemia and viremia (Blutt *et al.*, 2007; Ramig, 2007). The clinical significance of RV extra-intestinal spread is not clear, although there have been a number of anecdotal reports suggesting its rare involvement in other disease symptomatologies. Most notably, there have been reports of RV infection affecting the central nervous system, leading to convulsions, aseptic meningitis, and/or encephalitis (Dickey *et al.*, 2009; Rath *et al.*, 2011).

Rotavirus Morbidity and Mortality

Nearly all children by 5 years of age have been infected by RV at least once, regardless whether they live in developed or developing countries (Velazquez, 2009). However, multiple infections in the early years of life are probably the norm. For example, a landmark study by Velazquez *et al* (1996) showed that 13% of Mexican children had undergone 5 RV infections by two years of age. As shown by this and other studies, the first RV infection is the one that is most likely to produce moderate-to-severe diarrhea disease. The incidence of moderate-to-severe diarrhea decreases with second infections, and third infections are typically asymptomatic. RVs associated with second infections have G/P-genotypes that typically differ from those causing the primary infection. For instance in the Velazquez study, G1 and G3 viruses were the most frequent cause of primary infections, while G2 viruses were the most frequent cause of second infections. Such results suggest that primary infections induce protective responses that are at least partially genotype specific. It is this possibility - that RV infections induce strong G-type-specific immune responses - that has provided the impetus for developing multivalent vaccines that include multiple viruses with differing G types (Christy *et al.*, 1988).

Globally, RV infections result in an estimated 23 million outpatient visits and 2.3 million hospitalizations each year (Parashar *et al.*, 2009; Parashar *et al.*, 2003). Based on an analysis

of 2008 data, RV infections are estimated to cause 453,000 deaths per year, representing 5% of all deaths of young children (Tate *et al.*, 2011a). Most of these deaths take place in developing countries, particularly those located in sub-Saharan Africa and Southeast Asia. In India alone, there are nearly 100,000 deaths each year from RV infections.

Rotavirus Vaccines

Two RV vaccines (RotaTeq and Rotarix) were licensed for use in various countries of the world, including the United States, beginning in 2004–2005 (Yen *et al.*, 2011b). The US Advisory Committee on Immunization Practices (ACIP) recommended RotaTeq in 2006 and Rotarix in 2008 for universal vaccination of infants in the United States (Cortese, 2009; Parashar, 2006). In 2009, the World Health Organization recommended the inclusion of RV vaccines into the national immunization programs of all countries, and strongly recommended the introduction of vaccines into countries where diarrheal deaths are responsible for >10% of mortality of children that are younger than 5 years of age (WHO, 2009). The World Health Organization and the GAVI Alliance have efforts underway to support the introduction of RV vaccine programs into countries that have a high incidence of RV mortality but that lack the infrastructure or financial resources to develop such programs themselves.

RotaTeq (Merck) is a live-attenuated pentavalent vaccine that is administered orally to infants at 2, 4 and 6 months of age (Heaton & Ciarlet, 2007). The vaccine contains five human-bovine reassortant viruses [W179-9 (G1P[5]), SC-2 (G2P[5]), WI78-8 (G3P[5]), BrB-9 (G4P[5]), and WI79-4 (G6P[8])]; these were generated by crossing the naturally attenuated bovine RV strain WC3 with five unique human RVs each contributing a G1, G2, G3, or G4 VP7 or P[8] VP4 gene to one of the vaccine viruses (Matthijnssens *et al.*, 2010b). The multivalent design of the vaccine was based on the principle that the protective responses of RV infection may be predominantly homotypic in nature, vis-à-vis, specific to the G/P type of the infecting virus (Kapikian *et al.*, 1996). In fact, early protection studies with individual animal RV strains [e.g., RRV (G3P[3])] indicated that these monovalent viruses were poorly effective in inducing heterotypic protection (Christy *et al.*, 1988). Because the globally dominant G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] viruses all contain at least one G or P genotype in common with the RotaTeq vaccine strains, the vaccine should be effective in providing protective responses to any of the globally dominant strains, as well as other less frequently seen strains (e.g., G12P[8] and G2P[6] viruses). In order for RotaTeq to protect against the potentially globally-emerging G12P[6] viruses, the vaccine must induce heterotypic responses.

Rotarix (GlaxoSmithKline) is a live-attenuated monovalent vaccine that is administered orally to infants at 2 and 4 months of age (O'Ryan, 2007). The sole component of the vaccine is the human G1P[8] virus RIX4414, which was derived by serial passage in cell culture of a virus recovered from the stool of an infected child (Ruiz-Palacios *et al.*, 2006). Given the presence of G1- and P[8]-specific proteins in RIX4414, immunization with Rotarix should induce homotypic protective responses that protect vaccinees against all the globally dominant viruses except for those that have G2P[4] genotypes. Several, but not all, reports have concluded that Rotarix is effective in preventing severe diarrhea caused by G2P[4] viruses by inducing heterotypic responses (Correia *et al.*, 2010; Gurgel *et al.*, 2009; Snelling *et al.*, 2011; Yen *et al.*, 2011a). Whether this cross-reactive response involves neutralizing antibodies to VP7 or VP4, antibodies to internal genes (VP6, NSP2), or cytotoxic T lymphocytes is not clear.

The introduction of RotaTeq in 2006 and Rotarix in 2008 into the US childhood vaccination program has resulted in substantial reductions in levels of RV-associated health care (Tate *et*

al., 2011b; Tate *et al.*, 2009). Before routine RV vaccination began, RV infections caused an estimated 20–60 deaths, 55,000 hospitalizations, 200,000 emergency room visits, and 400,000 outpatient visits each year in the US, at a medical treatment cost of $300 million. During the January–June months of 2007–08 and 2008–09, RV-associated patient hospitalizations were reduced by an estimated 60–75%, at a cost savings of approximately $278 million (Cortes *et al.*, 2011). Other developed countries that have introduced RV vaccines into their immunization programs have seen similar successes in reducing the incidence of severe RV disease (Patel *et al.*, 2011). These outcomes are consistent with the results of clinical trials, which indicated an efficacy for the Rotarix and RotaTeq vaccines against severe gastroenteritis of at least 85% (Yen *et al.*, 2011b). In contrast, clinical trials have indicated that the vaccines are much less efficacious in some low-income countries, for reasons that are not fully understood (Armah *et al.*, 2010; Patel *et al.*, 2011; Zaman *et al.*, 2010).

Rotavirus Genomics and Evolution

The field of RV genomics originated in 2008 with publications comparing the genomes of 45 RVs, of which 25 were human strains (Heiman *et al.*, 2008; Matthijnssens *et al.*, 2008a). The analysis included the genome sequences of human RVs with 15 different G/P combinations (G1P[8], G2P[4], G3P[8], G3P[9], G4P[6], G5P[8], G6P[9], G8P[6], G8P[10], G9P[8], G10P[14], G12P[4], G12P[6], G12P[8], and G12P[9]). From these sequences, a complete genome classification system was developed for the RVs that allowed assignment of a genotype to each of the eleven viral genes (Matthijnssens *et al.*, 2008a). The 2008 studies provided evidence that the human RV strains, with few exceptions, had internal gene constellations that consisted entirely of either genotype 1 or genotype 2 genes (Heiman *et al.*, 2008; Matthijnssens *et al.*, 2008a). These results support the idea that human RVs have evolutionarily maintained preferred gene constellations, perhaps due to selective pressures favoring maintenance of viral protein sets that work ideally in virus replication. For example, the need for compatibility at protein-protein interfaces may explain why G1 VP7 is almost always a component of viruses with P[8] VP4 and genotype 1 VP6 proteins, while G2 VP7 is almost always a component of viruses with P[4] and genotype 2 VP6 proteins (see Figure 1C). Thus, although RVs can exchange genes through reassortment, the cost in most cases is probably to create a virus that is less evolutionarily fit than its parental viruses, rendering the reassortant unlikely to succeed in expanding into the landscape of circulating viruses.

An important outcome of the 2008 genomics studies was to provide the information necessary to design primer sets for sequencing the complete genomes of genogroup 1 and 2 viruses. Subsequently, these primer sets were used in generating a high-throughput RT-PCR sequencing pipeline at the J. Craig Venter Institute (JCVI). From this pipeline, the first large-scale sequencing project for the RVs was completed in 2009 (McDonald *et al.*, 2009). This analysis determined the genome sequences of 51 G3P[8] viruses in an archival stool collection recovered from children at Children's Hospital National Medical Center, Washington, D.C., from 1974–1991. A parallel project examining the G4P[8] viruses in the archival collection was published in 2011 (McDonald *et al.*, 2011), with an analysis of the G1P[8] viruses in the collection underway. In addition, the JCVI pipeline is being used to examine large contemporary collections of RVs recovered in the United States, Australia, and Belgium.

Intra-genotype alleles and virus clades

A goal of the genomics program has been to understand the relationship between RVs circulating over a period of time. For example, are all the viruses of the same G/P type genetically identical? And what is the genetic relationship between viruses that are of

different G/P types but belong to the same genogroup? Some insight into these questions has been provided by phylogenetic analysis of the genes of the G3P[8] and G4P[8] viruses circulating at DC Children's Hospital (McDonald *et al.*, 2011; McDonald *et al.*, 2009). The analysis showed that although all the genes were of genotype 1, the genes could be readily separated into several sub-genotype alleles. In addition, the analysis showed that some G3P[8] viruses and some G4P[8] viruses maintained different allele-based gene constellations, even though the viruses were collected from the same epidemic season. These findings indicate that distinct clades of viruses with the same G/P type can co-circulate in the same season. The fact that only minimal evidence of reassortment was noted between the clades, even those of the same G/P type, suggests that even at the sub-genotype level, there are selective pressures that favor maintenance of certain gene sets.

The DC Children's Hospital data also indicated that the number of virus clades circulating in a RV season can vary and that some clades will disappear from one season to the next, only to reappear years later. For example, three G3P[8] clades co-circulated in 1976 at DC Children's Hospital, but only one of these showed a close genetic relationship to the single major G3P[8] clade that circulated in 1991 (McDonald *et al.*, 2009). Comparison of G3P[8] and G4P[8] clades that co-circulated in 1990 demonstrated that some shared closely-related sub-genotype alleles; thus, these clades were likely linked through an earlier G3/G4 VP7 reassortment event (McDonald *et al.*, 2011). However, the 1990 G4P[8] clades also included some with sub-genotype constellations that consisted entirely of unique alleles. These results are again supportive of the concept that selective pressures brought about by the co-evolution of gene sets have provided a counter weight against the unlimited exchange of RV genes through reassortment.

Reassortment and antigenic variation

A crucial factor in the generation of reassortant viruses is the frequency of co-infection. In developing countries, the rate of RV co-infection can be as high as 20%, while in developed countries, the rate is typically less than 5% (Iturriza-Gomara & Gray, 2011; WHO, 2010). It may be because of the high rate of co-infection that the genetic diversity of viruses in developing countries can be so much higher than in developed countries. In developing countries, selective pressures that favor the maintenance of preferred gene constellation may be overwhelmed by the constant reshuffling of genes through co-infection and reassortment. This may explain reports describing the isolation of human RVs in developing countries that lack the complete genotype 1 or 2 constellations typical of common global G/P type viruses. Due to the high frequency of co-infection, large genetically distinct RV clades may not be detectable in some developing countries.

Sequence analysis has shown that the antigenic epitopes of VP7 and VP4 proteins assigned to the same G and P type, respectively, will frequently show amino acid variation (Jin *et al.*, 1996; McDonald *et al.*, 2009; Wu *et al.*, 2011; Zeller *et al.*, 2011). This has been seen for VP7 and VP4 proteins of viruses recovered from different countries in the same year or that belong to different co-circulating clades at one site. Such amino acid variation may ultimately have an impact on vaccine efficacy, particularly if protection is based chiefly on G and P type specific homotypic responses. In fact, Hoshino *et al.* (2005) have shown that the effective titer of a G type specific neutralizing antiserum is affected by the amino acid composition of VP7 antigenic epitopes, even if the VP7 proteins are of the same G type.

Vaccine Impact

Whether the widespread use of RV vaccines will have an impact on the diversity of evolution of human RVs cannot be fully accessed at this time, as most vaccination programs were established relatively recently (Kirkwood, 2010; Matthijnssens *et al.*, 2009; Patel *et al.*,

2011). Since selective pressures coming from the vaccine may be subtle, it could be many years before they are apparent. However, a recent analysis of children with RV diarrhea in Nicaragua, a country that exclusively vaccinates with RotaTeq, showed that two children shed G1P[8] viruses that contained an NSP2 gene identical in sequence to that of RotaTeq (Bucardo *et al.*, 2012). Otherwise the G1P[8] viruses were like other circulating G1P[8] viruses. This finding suggests that genome segments in the RV vaccine viruses will be introduced into the circulating pool of human viruses through reassortment.

Summary

Human RVs have specialized gene constellations that enable their efficient replication in the human host. Differences in their VP7 and VP4 proteins divide the human RVs into a defined number of antigenically distinct G/P types, some that are common globally and some that are only regionally important. Variations within the antigenic domains of the VP7 and VP4 proteins of the same G and P type, respectively, are common. Large-scale sequencing projects indicate that not only are human RVs antigenically diverse, but their genetic material continues to evolve. These sequencing projects also indicate that the temporal and geographical fluctuation of RVs results not from the dynamics of individual viral genes but from the behavior of genetically distinct RV clades.

Perhaps the most pressing issue in predicting the long-range effectiveness of RV vaccines is the lack of complete information concerning the immunologic basis of protection (Angel *et al.*, 2007; Velazquez, 2009). In particular, whether the vaccines are effective inducers of homotypic and/or broad heterotypic responses in immunized children are critical for predicting how well the vaccines will work against uncommon regional RV strains. Continued surveillance of circulating viruses in countries using RotaTeq and/or Rotarix should do much to clarify the mechanism by which these vaccines work.

The RV vaccines face quite a challenge. While they are composed of genetically fixed viruses that grow poorly in humans, the viruses that they are up against are genetically diverse and evolutionarily dynamic and can be shed into the environment at high titers by the ill child. From a molecular vantage point, it will be a remarkable feat if the vaccine strains can hold dominance over the landscape of diverse, evolving viruses to prevent the emergence of new strains that are unaffected by current vaccines. Certainly, it would make for a fine second act to the battle of David and Goliath.

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References

Anderson EJ, Weber SG. Rotavirus infection in adults. Lancet Infect Dis. 2004; 4(2):91–99. [PubMed: 14871633]

Angel J, Franco MA, Greenberg HB. Rotavirus vaccines: recent developments and future considerations. Nat Rev Microbiol. 2007; 5(7):529–539. [PubMed: 17571094]

Aoki ST, Settembre EC, Trask SD, Greenberg HB, Harrison SC, Dormitzer PR. Structure of rotavirus outer-layer protein VP7 bound with a neutralizing Fab. Science. 2009; 324(5933):1444–1447. [PubMed: 19520960]

Arias CF, Romero P, Alvarez V, Lopez S. Trypsin activation pathway of rotavirus infectivity. J Virol. 1996; 70(9):5832–5839. [PubMed: 8709201]

Armah GE, Steele AD, Esona MD, Akran VA, Nimzing L, Pennap G. Diversity of rotavirus strains circulating in west Africa from 1996 to 2000. J Infect Dis. 2010; 202(Suppl):S64–S71. [PubMed: 20684720]

Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, Binka FN, Steele AD, Laserson KF, Ansah NA, Levine MM, Lewis K, Coia ML, Attah-Poku M, Ojwando J, Rivers SB, Victor JC, Nyambane G, Hodgson A, Schödel F, Ciarlet M, Neuzil KM. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet. 2010; 376(9741):606–614. [PubMed: 20692030]

Attoui, H.; Mertens, PPC.; Becnel, J.; Belaganahalli, S.; Bergoin, M.; Brussaard, CP.; Chappell, JD.; Ciarlet, M.; del Vas, M.; Dermody, TS.; Dormitzer, PR.; Duncan, R.; Fcang, Q.; Graham, R.; Guglielmi, KM.; Harding, RM.; Hillman, B.; Makkay, A.; Marzachì, C.; Matthijnssens, J., et al. Reoviridae. In: King, A.; Adams, M.; Carstens, E.; Lefkwitz, E., editors. Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press; Waltham, MA, USA: 2012. p. 497-650.

Bernstein DI. Rotavirus overview. Pediatr Infect Dis J. 2009; 28(3 Suppl):S50–S53. [PubMed: 19252423]

Binka E, Vermund SH, Armah GE. Rotavirus diarrhea among children less than 5 years of age in urban Ghana. Pediatr Infect Dis J. 2011; 30(8):716–718. [PubMed: 21646938]

Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet. 1973; 2(7841):1281–1283. [PubMed: 4127639]

Blutt SE, Matson DO, Crawford SE, Staat MA, Azimi P, Bennett BL, Piedra PA, Conner ME. Rotavirus antigenemia in children is associated with viremia. PLoS Med. 2007; 4(4):e121. [PubMed: 17439294]

Bucardo F, Rippinger CM, Svensson L, Patton JT. Rotaviruses from vaccinated Nicaraguan children with gastroenteritis contain a RotaTeq-derived genome segment. Infection, Genetics, and Evolution. 2012 submitted.

Caul EO, Ashley CR, Darville JM, Bridger JC. Group C rotavirus associated with fatal enteritis in a family outbreak. J Med Virol. 1990; 30(3):201–205. [PubMed: 2341836]

Christy C, Madore HP, Pichichero ME, Gala C, Pincus P, Vosefski D, Hoshino Y, Kapikian A, Dolin R. Field trial of rhesus rotavirus vaccine in infants. Pediatr Infect Dis J. 1988; 7(9):645–650. [PubMed: 2845349]

Clark HF, Lawley DA, Schaffer A, Patacsil JM, Marcello AE, Glass RI, Jain V, Gentsch J. Assessment of the epidemic potential of a new strain of rotavirus associated with the novel G9 serotype which caused an outbreak in the United States for the first time in the 1995–1996 season. J Clin Microbiol. 2004; 42(4):1434–1438. [PubMed: 15070985]

Cook SM, Glass RI, Lebaron CW, Ho MS. Global seasonality of rotavirus infections. Bull World Health Organ. 1990; 68(2):171–177. [PubMed: 1694734]

Correia JB, Patel MM, Nakagomi O, Montenegro FM, Germano EM, Correia NB, Cuevas LE, Parashar UD, Cunliffe NA, Nakagomi T. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. J Infect Dis. 2010; 201(3):363–369. [PubMed: 20047501]

Cortes JE, Curns AT, Tate JE, Cortese MM, Patel MM, Zhou F, Parashar UD. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. N Engl J Med. 2011; 365(12):1108–1117. [PubMed: 21992123]

Cortese M, Parashar UD. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committe on Immunization Practices (ACIP). MMWR Recomm Rep, Centers for Disease Control and Prevention. 2009; 58(RR02):1–25.

Coulson B. VP4 and VP7 typing using monoclonal antibodies. Arch Virol Suppl. 1996; 12:113–118. [PubMed: 9015108]

Cunliffe NA, Dove W, Bunn JE, Ben Ramadam M, Nyangao JW, Riveron RL, Cuevas LE, Hart CA. Expanding global distribution of rotavirus serotype G9: detection in Libya, Kenya, and Cuba. Emerg Infect Dis. 2001; 7(5):890–892. [PubMed: 11747706]

Dickey M, Jamison L, Michaud L, Care M, Bernstein DI, Staat MA. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. Pediatr Infect Dis J. 2009; 28(4):318–321. [PubMed: 19258926]

Dormitzer PR, Sun ZY, Wagner G, Harrison SC. The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. EMBO J. 2002; 21(5):885–897. [PubMed: 11867517]

Esona MD, Banyai K, Foytich K, Freeman M, Mijatovic-Rustempasic S, Hull J, Kerin T, Steele AD, Armah GE, Geyer A, Page N, Agbaya VA, Forbi JC, Aminu M, Gautam R, Seheri LM, Nyangao J, Glass R, Bowen MD, Gentsch JR. Genomic characterization of human rotavirus G10 strains from the African Rotavirus Network: relationship to animal rotaviruses. Infect Genet Evol. 2011; 11(1):237–241. [PubMed: 20934537]

Fang ZY, Ye Q, Ho MS, Dong H, Qing S, Penaranda ME, Hung T, Wen L, Glass RI. Investigation of an outbreak of adult diarrhea rotavirus in China. J Infect Dis. 1989; 160(6):948–953. [PubMed: 2555422]

Feng N, Kim B, Fenaux M, Nguyen H, Vo P, Omary MB, Greenberg HB. Role of interferon in homologous and heterologous rotavirus infection in the intestines and extraintestinal organs of suckling mice. J Virol. 2008; 82(15):7578–7590. [PubMed: 18495762]

Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992; 30(6): 1365–1373. [PubMed: 1320625]

Ghosh S, Kobayashi N. Whole-genomic analysis of rotavirus strains: current status and future prospects. Future Microbiol. 2011; 6:1049–1065. [PubMed: 21958144]

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol. 1990; 28(2):276–282. [PubMed: 2155916]

Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. Gastroenterology. 2009; 136(6):1939–1951. [PubMed: 19457420]

Gurgel RG, Bohland AK, Vieira SC, Oliveira DM, Fontes PB, Barros VF, Ramos MF, Dove W, Nakagomi T, Nakagomi O, Correia JB, Cunliffe N, Cuevas LE. Incidence of rotavirus and all-cause diarrhea in northeast Brazil following the introduction of a national vaccination program. Gastroenterology. 2009; 137(6):1970–1975. [PubMed: 19632228]

Heaton PM, Ciarlet M. Vaccines: the pentavalent rotavirus vaccine: discovery to licensure and beyond. Clin Infect Dis. 2007; 45(12):1618–1624. [PubMed: 18198497]

Heiman EM, McDonald SM, Barro M, Taraporewala ZF, Bar-Magen T, Patton JT. Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. J Virol. 2008; 82(22):11106–11116. [PubMed: 18786998]

Hoshino Y, Honma S, Jones RW, Ross J, Santos N, Gentsch JR, Kapikian AZ, Hesse RA. A porcine G9 rotavirus strain shares neutralization and VP7 phylogenetic sequence lineage 3 characteristics with contemporary human G9 rotavirus strains. Virology. 2005; 332(1):177–188. [PubMed: 15661150]

Iturriza-Gomara, M.; Gray, J. 4th Year Report. 2011. EuroRotaNet.

Iturriza-Gomara M, Dallman T, Banyai K, Bottiger B, Buesa J, Diedrich S, Fiore L, Johansen K, Koopmans M, Korsun N, Koukou D, Kroneman A, Laszlo B, Lappalainen M, Maunula L, Marques AM, Matthijnssens J, Midgley S, Mladenova Z, Nawaz S, et al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. Epidemiol Infect. 2011; 139(6):895–909. [PubMed: 20707941]

Jere KC, Mlera L, O'Neill HG, Potgieter AC, Page NA, Seheri ML, Van Dijk AA. Whole genome analyses of African G2, G8, G9, and G12 rotavirus strains using sequence-independent amplification and 454(R) pyrosequencing. J Med Virol. 2011a; 83(11):2018–2042. [PubMed: 21915879]

Jere KC, Sawyerr T, Seheri LM, Peenze I, Page NA, Geyer A, Steele AD. A first report on the characterization of rotavirus strains in Sierra Leone. J Med Virol. 2011b; 83(3):540–550. [PubMed: 21264877]

Jin Q, Ward RL, Knowlton DR, Gabbay YB, Linhares AC, Rappaport R, Woods PA, Glass RI, Gentsch JR. Divergence of VP7 genes of G1 rotaviruses isolated from infants vaccinated with reassortant rhesus rotaviruses. Arch Virol. 1996; 141(11):2057–2076. [PubMed: 8973523]

Kapikian AZ, Hoshino Y, Chanock RM, Perez-Schael I. Jennerian and modified Jennerian approach to vaccination against rotavirus diarrhea using a quadrivalent rhesus rotavirus (RRV) and human-RRV reassortant vaccine. Arch Virol Suppl. 1996; 12:163–175. [PubMed: 9015113]

Estes, MK.; Kapikian, A. Rotaviruses. In: Knipe, D.; Griffin, D.; Lamb, R.; Martin, M.; Roizman, B.; Straus, S., editors. Fields Virology. Wolters Kluwer Health; Lippincott, Williams and Wilkins; Philadelphia, PA, USA: 2007. p. 1917-1975.

Kirkwood CD. Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. J Infect Dis. 2010; 202(Suppl):S43–S48. [PubMed: 20684716]

Liu M, Mattion NM, Estes MK. Rotavirus VP3 expressed in insect cells possesses guanylyltransferase activity. Virology. 1992; 188(1):77–84. [PubMed: 1314468]

Lu X, McDonald SM, Tortorici MA, Tao YJ, Vasquez-Del Carpio R, Nibert ML, Patton JT, Harrison SC. Mechanism for coordinated RNA packaging and genome replication by rotavirus polymerase VP1. Structure. 2008; 16(11):1678–1688. [PubMed: 19000820]

Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. Vet Microbiol. 2010; 140(3–4):246–255. [PubMed: 19781872]

Matsumoto K, Hatano M, Kobayashi K, Hasegawa A, Yamazaki S, Nakata S, Chiba S, Kimura Y. An outbreak of gastroenteritis associated with acute rotaviral infection in schoolchildren. J Infect Dis. 1989; 160(4):611–615. [PubMed: 2551975]

Matthijnssens J, Bilcke J, Ciarlet M, Martella V, Banyai K, Rahman M, Zeller M, Beutels P, Van Damme P, Van Ranst M. Rotavirus disease and vaccination: impact on genotype diversity. Future Microbiol. 2009; 4(10):1303–1316. [PubMed: 19995190]

Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, Mcdonald SM, Palombo EA, Iturriza-Gomara M, Maes P, Patton JT, Rahman M, Van Ranst M. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol. 2008a; 82(7):3204–3219. [PubMed: 18216098]

Matthijnssens J, Ciarlet M, Mcdonald SM, Attoui H, Banyai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gomara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreno V, Rahman M, Ruggeri FM, Saif LJ, et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol. 2011a; 156(8): 1397–1413. [PubMed: 21597953]

Matthijnssens J, De Grazia S, Piessens J, Heylen E, Zeller M, Giammanco GM, Banyai K, Buonavoglia C, Ciarlet M, Martella V, Van Ranst M. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. Infect Genet Evol. 2011b; 11(6):1396–1406. [PubMed: 21609783]

Matthijnssens J, Heylen E, Zeller M, Rahman M, Lemey P, Van Ranst M. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. Mol Biol Evol. 2010a; 27(10):2431–2436. [PubMed: 20522727]

Matthijnssens J, Joelsson DB, Warakomski DJ, Zhou T, Mathis PK, Van Maanen MH, Ranheim TS, Ciarlet M. Molecular and biological characterization of the 5 human-bovine rotavirus (WC3)-based reassortant strains of the pentavalent rotavirus vaccine, RotaTeq. Virology. 2010b; 403(2): 111–127. [PubMed: 20451234]

Matthijnssens J, Mino S, Papp H, Potgieter C, Novo L, Heylen E, Zeller M, Garaicoeachea L, Badaracco A, Lengyel G, Kisfali P, Collins P, Ciarlet M, O'Shea H, Parreno V, Banyai K, Barrandeguy M, Van Ranst M. Complete molecular genome analyses of equine rotavirus A strains from different continents reveal several new genotypes and a largely conserved genotype constellation. J Gen Virol. 2011c in press.

Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6 sequence-based cut-off values as a criterion for rotavirus species demarcation. Arch Virol. 2012 in press.

Matthijnssens J, Rahman M, Van Ranst M. Two out of the 11 genes of an unusual human G6P[6] rotavirus isolate are of bovine origin. J Gen Virol. 2008b; 89(Pt 10):2630–2635. [PubMed: 18796733]

McClain B, Settembre E, Temple BR, Bellamy AR, Harrison SC. X-ray crystal structure of the rotavirus inner capsid particle at 3.8 A resolution. J Mol Biol. 2010; 397(2):587–599. [PubMed: 20122940]

McDonald SM, Davis K, Mcallen JK, Spiro DJ, Patton JT. Intra-genotypic diversity of archival G4P[8] human rotaviruses from Washington, DC. Infect Genet Evol. 2011; 11(7):1586–1594. [PubMed: 21712102]

McDonald SM, Matthijnssens J, Mcallen JK, Hine E, Overton L, Wang S, Lemey P, Zeller M, Van Ranst M, Spiro DJ, Patton JT. Evolutionary dynamics of human rotaviruses: balancing reassortment with preferred genome constellations. PLoS Pathog. 2009; 5(10):e1000634. [PubMed: 19851457]

Muller H, Johne R. Rotaviruses: diversity and zoonotic potential--a brief review. Berl Munch Tierarztl Wochenschr. 2007; 120(3–4):108–112. [PubMed: 17416132]

O'Ryan M. Rotarix (RIX4414): an oral human rotavirus vaccine. Expert Rev Vaccines. 2007; 6(1):11–19. [PubMed: 17280473]

O'Ryan M. The ever-changing landscape of rotavirus serotypes. Pediatr Infect Dis J. 2009; 28(3 Suppl):S60–S62. [PubMed: 19252426]

Parashar U, Alexander JP, Glass RI. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committe on Immunization Practices (ACIP). MMWR Recomm Rep. 2006; 55(RR-12):1–13. [PubMed: 16902398]

Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis. 2009; 200(Suppl 1):S9–S15. [PubMed: 19817620]

Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis. 2003; 9(5):565–572. [PubMed: 12737740]

Park SI, Matthijnssens J, Saif LJ, Kim HJ, Park JG, Alfajaro MM, Kim DS, Son KY, Yang DK, Hyun BH, Kang MI, Cho KO. Reassortment among bovine, porcine and human rotavirus strains results in G8P[7] and G6P[7] strains isolated from cattle in South Korea. Vet Microbiol. 2011; 152(1–2): 55–66. [PubMed: 21592683]

Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, Parashar UD. Real-world impact of rotavirus vaccination. Pediatr Infect Dis J. 2011; 30(1 Suppl):S1–5. [PubMed: 21183833]

Payne DC, Staat MA, Edwards KM, Szilagyi PG, Gentsch JR, Stockman LJ, Curns AT, Griffin M, Weinberg GA, Hall CB, Fairbrother G, Alexander J, Parashar UD. Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. Pediatrics. 2008; 122(6):1235–1243. [PubMed: 19047240]

Payne DC, Staat MA, Edwards KM, Szilagyi PG, Weinberg GA, Hall CB, Chappell J, Curns AT, Wikswo M, Tate JE, Lopman BA, Parashar UD. Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US Counties, 2006–2009. Clin Infect Dis. 2011; 53(3):245–253. [PubMed: 21705316]

Payne DC, Szilagyi PG, Staat MA, Edwards KM, Gentsch JR, Weinberg GA, Hall CB, Curns AT, Clayton H, Griffin MR, Fairbrother G, Parashar UD. Secular variation in United States rotavirus disease rates and serotypes: implications for assessing the rotavirus vaccination program. Pediatr Infect Dis J. 2009; 28(11):948–953. [PubMed: 19859013]

Rahman M, Sultana R, Ahmed G, Nahar S, Hassan ZM, Saiada F, Podder G, Faruque AS, Siddique AK, Sack DA, Matthijnssens J, Van Ranst M, Azim T. Prevalence of G2P[4] and G12P[6] rotavirus, Bangladesh. Emerg Infect Dis. 2007; 13(1):18–24. [PubMed: 17370511]

Ramani S, Sankaran P, Arumugam R, Sarkar R, Banerjee I, Mohanty I, Jana AK, Kuruvilla KA, Kang G. Comparison of viral load and duration of virus shedding in symptomatic and asymptomatic neonatal rotavirus infections. J Med Virol. 2010; 82(10):1803–1807. [PubMed: 20827780]

Ramig RF. Systemic rotavirus infection. Expert Rev Anti Infect Ther. 2007; 5(4):591–612. [PubMed: 17678424]

Rath BA, Gentsch J, Seckinger J, Ward K, Deputy S. Rotavirus Encephalitis With Basal Ganglia Involvement in an 8-Month-Old Infant. Clin Pediatr (Phila). 2011

Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, Lopez P, Macias-Parra M, Ortega-Barria E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavia-Ruz N, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006; 354(1):11–22. [PubMed: 16394298]

Samajdar S, Varghese V, Barman P, Ghosh S, Mitra U, Dutta P, Bhattacharya SK, Narasimham MV, Panda P, Krishnan T, Kobayashi N, Naik TN. Changing pattern of human group A rotaviruses: emergence of G12 as an important pathogen among children in eastern India. J Clin Virol. 2006; 36(3):183–188. [PubMed: 16679056]

Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol. 2005; 15(1): 29–56. [PubMed: 15484186]

Settembre EC, Chen JZ, Dormitzer PR, Grigorieff N, Harrison SC. Atomic model of an infectious rotavirus particle. EMBO J. 2011; 30(2):408–416. [PubMed: 21157433]

Snelling TL, Andrews RM, Kirkwood CD, Culvenor S, Carapetis JR. Case-control evaluation of the effectiveness of the G1P[8] human rotavirus vaccine during an outbreak of rotavirus G2P[4] infection in central Australia. Clin Infect Dis. 2011; 52(2):191–199. [PubMed: 21288843]

Steele AD, Ivanoff B. Rotavirus strains circulating in Africa during 1996–1999: emergence of G9 strains and P[6] strains. Vaccine. 2003; 21(5–6):361–367. [PubMed: 12531633]

Steele AD, Peenze I, De Beer MC, Pager CT, Yeats J, Potgieter N, Ramsaroop U, Page NA, Mitchell JO, Geyer A, Bos P, Alexander JJ. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. Vaccine. 2003; 21(5–6):354–360. [PubMed: 12531632]

Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 2011a in press.

Tate JE, Mutuc JD, Panozzo CA, Payne DC, Cortese MM, Cortes JE, Yen C, Esposito DH, Lopman BA, Patel MM, Parashar UD. Sustained decline in rotavirus detections in the United States following the introduction of rotavirus vaccine in 2006. Pediatr Infect Dis J. 2011b; 30(1 Suppl):S30–S34. [PubMed: 21183838]

Tate JE, Panozzo CA, Payne DC, Patel MM, Cortese MM, Fowlkes AL, Parashar UD. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. Pediatrics. 2009; 124(2):465–471. [PubMed: 19581260]

Trojnar E, Otto P, Roth B, Reetz J, Johne R. The genome segments of a group D rotavirus possess group A-like conserved termini but encode group-specific proteins. J Virol. 2010; 84(19):10254–10265. [PubMed: 20631147]

Velazquez FR. Protective effects of natural rotavirus infection. Pediatr Infect Dis J. 2009; 28(3 Suppl):S54–S56. [PubMed: 19252424]

Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, Glass RI, Estes MK, Pickering LK, Ruiz-Palacios GM. Rotavirus infections in infants as protection against subsequent infections. N Engl J Med. 1996; 335(14):1022–1028. [PubMed: 8793926]

Vesikari T. Trials of oral bovine and rhesus rotavirus vaccines in Finland: a historical account and present status. Arch Virol Suppl. 1996; 12:177–186. [PubMed: 9015114]

Vesikari T, Kapikian AZ, Delem A, Zissis G. A comparative trial of rhesus monkey (RRV-1) and bovine (RIT 4237) oral rotavirus vaccines in young children. J Infect Dis. 1986; 153(5):832–839. [PubMed: 3009634]

WHO. Rotavirus vaccines: an update. World Health Organization, Weekly Epidemiological Record. 2009; 84:533–540.

WHO. Global rotavirus information and surveillance bulletin. Reporting period: January through December 2010. Vol. 4. World Health Organization; 2011.

Wu FT, Banyai K, Huang JC, Wu HS, Chang FY, Yang JY, Hsiung CA, Huang YC, Lin JS, Hwang KP, Jiang B, Gentsch JR. Diverse origin of P[19] rotaviruses in children with acute diarrhea in

Taiwan: Detection of novel lineages of the G3, G5, and G9 VP7 genes. J Med Virol. 2011; 83(7): 1279–1287. [PubMed: 21567431]

Yen C, Figueroa JR, Uribe ES, Carmen-Hernandez LD, Tate JE, Parashar UD, Patel MM, Richardson Lopez-Collado V. Monovalent rotavirus vaccine provides protection against an emerging fully heterotypic G9P[4] rotavirus strain in Mexico. J Infect Dis. 2011a; 204(5):783–786. [PubMed: 21810915]

Yen C, Tate JE, Patel MM, Cortese MM, Lopman B, Fleming J, Lewis K, Jiang B, Gentsch J, Steele D, Parashar UD. Rotavirus vaccines: Update on global impact and future priorities. Hum Vaccin. 2011b; 7(12) in press.

Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ, Podder G, Vu DT, Le TP, Luby SP, Le HT, Coia ML, Lewis K, Rivers SB, Sack DA, Schödel F, Steele AD, Neuzil KM, Ciarlet M. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet. 2010; 376(9741):615–623. [PubMed: 20692031]

Zeller M, Patton JT, Heylen E, Decoster S, Ciarlet M, Van Ranst M, Matthijnssens J. Genetic analyses reveal differences in the VP7 and VP4 antigenic epitopes between human rotaviruses circulating in Belgium and rotaviruses in Rotarix and Rotateq. J Clin Microbiol. 2011 in press.

Zuridah H, Kirkwood CD, Bogdanovic-Sakran N, Bishop RF, Yap KL. Circulating human group A rotavirus genotypes in Malaysia. J Med Virol. 2010; 82(4):707–711. [PubMed: 20166178]

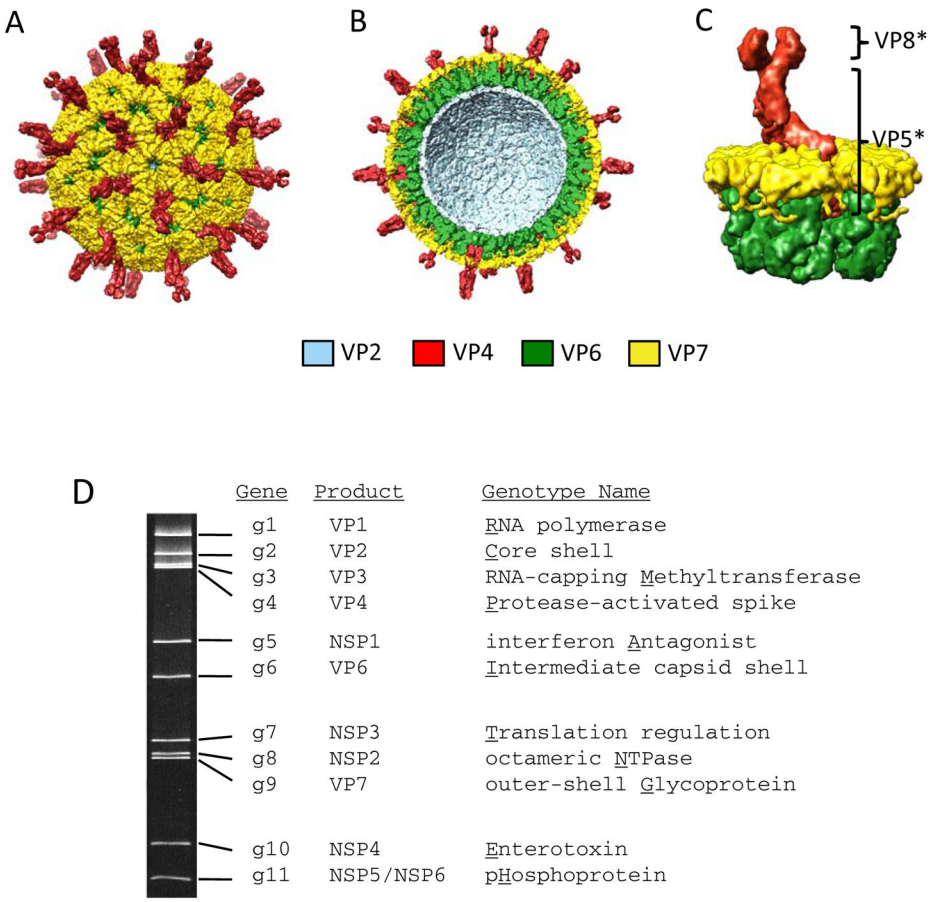

Figure 1.

Rotavirus capsid structure and dsRNA genome. (A) Intact triple-layered virion with VP4 spikes projecting from the VP7 outer capsid shell. (B) Cut-away of virion revealing the three protein layers of the virion: VP2, VP6, and VP7. Note that the foot of the VP4 spike extends into the VP6 layer. (C) A VP6 hexamer, VP7 hexamer, and embedded VP4 spike, with the VP8* and VP5* regions of VP4 identified. (D) Double-stranded RNA segments of the RV genome resolved by gel electrophoresis. Segments are labeled as g1-g11 (g = gene), and their protein products are listed. Associated functions or properties of the protein products are given (Genotype name). The underlined letter identifies the segment in the gene constellation acronym: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx.

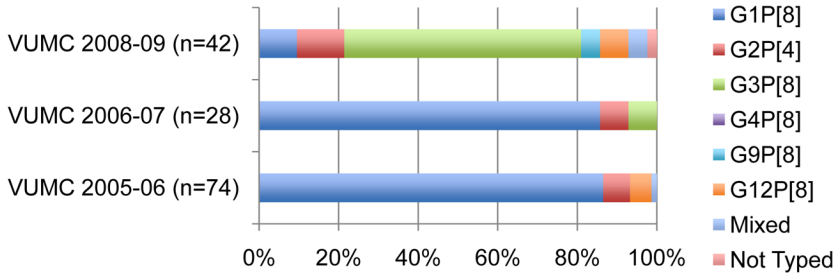

Figure 2.
G/P-genotypes of RVs recovered from children with gastroenteritis at Vanderbilt University Medical Center during three winter-spring seasons. Sample number (n).

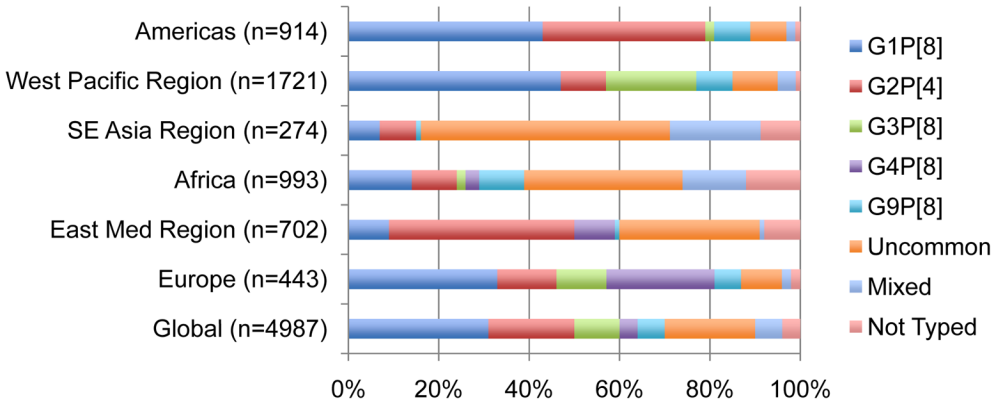

Figure 3.
Distribution of RV Genotypes Reported to the WHO Surveillance Network in 2010. Sample number (n).

Table 1

Genotypes of human, animal and vaccine RVs.[1]

			Genotype (# possible)										
			VP7 (27)	**VP4 (35)**	**VP6 (16)**	**VP1 (9)**	**VP2 (9)**	**VP3 (8)**	**NSP1 (16)**	**NSP2 (9)**	**NSP3 (12)**	**NSP4 (14)**	**NSP5 (11)**
Common Global Strains	Wa	human	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	DS-1	human	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
	P	human	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	ST3	human	G4	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	WI61	human	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
Vaccine Strains	Rotarix RIX441	human	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RotaTeq WI79-9	Hu x Bo reassort	G1	P[5]	I2	R2	C2	M1	A3	N2	T6	E2	H3
	RotaTeq SC2-9	Hu x Bo reassort	G2	P[5]	I2	R2	C2	M1	A3	N2	T6	E2	H3
	RotaTeq WI78-8	Hu x Bo reassort	G3	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
	RotaTeq BrB-9	Hu x Bo reassort	G4	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
	RotaTeq WI79-4	Hu x Bo reassort	G6	P[8]	I2	R2	C2	M2	A3	N2	T6	E2	H3
Uncommon Strains	Au-1	human	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
	MWI	human	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
	GR 10924/99	human	G9	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
	6717/2002/ARN	human	G10	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	Dhaka12-03	human	G12	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	Dhaka25-02	human	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
Animal Strains	SA11-H96	simian	G3	P[2]	I3	R2	C5	M5	A5	N5	T5	E2	H5
	RRV	simian	G8	P[3]	I2	R2	C3	M3	A9	N2	T3	E3	H6
	OSU	porcine	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1

			Genotype (# possible)									
			VP7 (27)	**VP4 (35)**	**VP6 (16)**	**VP1 (9)**	**VP2 (9)**	**VP3 (8)**	**NSP1 (16)**	**NSP2 (9)**	**NSP3 (12)**	**NSP4 (14)**	**NSP5 (11)**
	WC3	bovine	G6	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3

[1] Genotype 1 and 2 genes have been colored green and red, respectively.