Published in final edited form as:

Infect Genet Evol. 2012 August ; 12(6): 1282–1294. doi:10.1016/j.meegid.2012.03.007.

Vaccine-Derived NSP2 Segment in Rotaviruses from Vaccinated Children with Gastroenteritis in Nicaragua

Filemón Bucardo¹, Christine M. Rippinger², Lennart Svensson³, and John T. Patton^{2,*}

¹Department of Microbiology, University of León, UNAN-León, Nicaragua

²Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

³Division of Molecular Virology, Department of Clinical and Experimental Medicine, Linköping University, Sweden

Abstract

Rotavirus (RV) vaccination programs have been established in several countries using the human-attenuated G1P[8] monovalent vaccine Rotarix™ (GlaxoSmithKline) and/or the human-bovine reassortant G1, G2, G3, G4, P[8] pentavalent vaccine RotaTeq™ (Merck). The efficacy of both vaccines is high (~90%) in developed countries, but can be remarkably lower in developing countries. For example, a vaccine efficacy against severe diarrhea of only 58% was observed in a 2007–2009 Nicaraguan study using RotaTeq. To gain insight into the significant level of vaccine failure in this country, we sequenced the genomes of RVs recovered from vaccinated Nicaraguan children with gastroenteritis. The results revealed that all had genotype specificities typical for human RVs (11 G1P[8], 1 G3P[8]) and that the sequences and antigenic epitopes of the outer capsid proteins (VP4 and VP7) of these viruses were similar to those reported for RVs isolated elsewhere in the world. As expected, nine of the G1P[8] viruses and the single G3P[8] virus had genome constellations typical of human G1P[8] and G3P[8] RVs: G1/3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. However, two of the G1P[8] viruses had atypical constellations, G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1, due to the presence of a genotype-2 NSP2 (N2) gene. The sequence of the N2 NSP2 gene was identical to the bovine N2 NSP2 gene of RotaTeq, indicating that the two atypical viruses originated via reassortment of human G1P[8] RVs with RotaTeq viruses. Together, our data suggest that the high level of vaccine failure in Nicaraguan is probably not due to antigenic drift of commonly circulating virus strains nor the emergence of new antigenetically distinct virus strains. Furthermore, our data suggests that the widespread use of the RotaTeq vaccine has led to the introduction of vaccine genes into circulating human RVs.

Keywords

rotavirus; genomics; vaccine; RotaTeq

*Corresponding author: John T. Patton, Laboratory of Infectious Diseases, Building 50, Room 6313, 50 South Drive, MSC8007, NIH, Bethesda, MD 20892. Phone: (301) 594-1615; Fax (301) 480-7941; jpatton@niaid.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Rotavirus (RV) is a major cause of severe potentially life-threatening diarrhea in infants and children under the age of 5 years (Parashar et al., 2009; Parashar et al., 2003). Globally, RV infections cause ~450,000 deaths each year in this age range, with the vast majority occurring in Sub-Saharan Africa and Southeast Asia (Tate et al., 2012). RVs belong to the family *Reoviridae* and have icosahedral multilayered capsids that contain eleven segments of double-stranded (ds)RNA (Estes and Kapikian, 2007). The outer capsid Glycoprotein VP7 and Protease-activated spike protein VP4 elicit neutralizing antibodies, and their antigenic and sequences properties have been used to define the G and P serotypes and genotypes, respectively, of RV strains (Aoki et al., 2009; Coulson, 1996; Dormitzer et al., 2002; Hoshino and Kapikian, 1996). In humans, RVs with G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] genotype specificities are responsible for most disease (Iturriza-Gomara et al., 2011; Santos and Hoshino, 2005; WHO, 2011). Recently, a classification system was developed that allows genotype assignment to all eleven RV genome segments (genes), with the acronym Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx representing the genotype designations of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5-NSP6 genes (Matthijnssens et al., 2011; Matthijnssens et al., 2008). Human RVs that cause disease typically have the genome constellation G1/3/4/9-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 (genogroup 1 viruses) or G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 (genogroup 2 viruses) (Heiman et al., 2008; Matthijnssens et al., 2008; McDonald et al., 2009).

The pentavalent RV vaccine RotaTeq™ (Merck) consists of five virus strains with the genotype specificities G1P[5], G2P[5], G3P[5], G4P[5], and G6P[8] (Matthijnssens et al., 2010). Each strain were generated by reassortment of a select human RV with WC3, a G6P[5] bovine RV that is attenuated in humans (Clark et al., 2006; Heaton and Ciarlet, 2007). A clinical trial conducted primarily in the US and Finland, involving 68,038 children, indicated that the efficacy of RotaTeq against G1 – G4 RV gastroenteritis of any severity was 74% and against severe RV gastroenteritis was 98% (Vesikari et al., 2006). In contrast, the efficacy of RotaTeq against severe RV gastroenteritis was 48% in clinical trials carried out in Bangladesh and Vietnam (Zaman et al., 2010), even though nearly all the participants with severe diarrhea in these studies were infected with RVs that had G-or P-type specificities included in RotaTeq (Vesikari et al., 2006; Zaman et al., 2010). Evaluation of RotaTeq in a 2007–2008 study in Nicaragua revealed that the vaccine had an efficacy against severe diarrhea of only 58% (Patel et al., 2009a). In this study, G2P[4] viruses were identified as the causative agent in 88% cases of disease. Because RotaTeq contains a G2 antigen but not a P[4] component, the possibility was raised that the vaccine might be less effective in protecting against G2P[4] RV diarrheal disease (Patel et al., 2009a).

In Brazil, a predominance of G2P[4] RV diarrheal disease was observed soon after the establishment of a national vaccination program using the monovalent G1P[8] RV vaccine, Rotarix™ (GlaxoSmithKline). This led to the speculation that Rotarix was less effective against G2P[4] RVs than P[8] non-G2 viruses and that Rotarix created selective conditions favoring the emergence of G2P[4] viruses as the primary cause of diarrheal disease (Gurgel et al., 2007; Nakagomi et al., 2008). However, recent RV surveillance information suggests that the increased incidence of G2P[4] viruses at the time of Rotarix introduction more likely resulted from a natural fluctuation of RV genotypes (Carvalho-Costa et al., 2011). Moreover, an efficacy assessment completed for Rotarix in children aged 6–11 months in Brazil showed that it reduced G2P[4] diarrheal disease by 77%, indicating that the vaccine was capable of inducing heterotypic protection (Correia et al., 2010). Likewise, a study of European children by Vesikari et al (2007) indicated that Rotarix provided significant protection against G2-associated disease. On the other hand, the results of a clinical trial carried out in Malawi showed that the efficacy of Rotarix against severe gastroenteritis

caused by non-G1 RVs was only 50% (Madhi et al., 2010). Thus, although the vaccine may induce heterotypic protection, the efficacy of Rotarix can be significantly decreased in some developing countries (Yen et al., 2011b).

Altogether, studies with RotaTeq and Rotarix indicate that a significant proportion of vaccinated children in some developing countries still experience severe RV diarrhea (WHO, 2009; Yen et al., 2011b). To what extent such vaccine failures result from infections with RVs that have an atypical or mutated genetic make-up that allow the viruses to circumvent vaccine-induced protection in young children is not known. However, given that amino acid differences at the antigenic sites on the RV outer capsid proteins VP7 and VP4 can affect the ability of antibodies to neutralize virus infectivity (Coulson and Kirkwood, 1991; Dyall-Smith et al., 1986; Hoshino et al., 2005), it is possible that mutations in the RV genome could undermine vaccine effectiveness.

In the current study, the genomes of RV strains isolated from diarrheic children fully or partially immunized with the RotaTeq vaccine were sequenced in order to identify possible unusual genome constellations, genotypes, or sequences that might account for the ability of these viruses to cause disease in vaccinated children. Analysis of these viruses showed that they were predominantly of G1P[8] specificity with neutralization domains in their VP7 and VP4 proteins similar to viruses circulating elsewhere in the world. Surprisingly, two of the G1P[8] viruses contained atypical genome constellations (G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1). The N2 NSP2 gene in these two viruses was identical to that of the bovine N2 NSP2 gene of RotaTeq, indicating that two of the vaccinated children with gastroenteritis had contracted infections with G1P[8] RVs that derived from reassortment of human virus strains with Rotateq vaccine viruses.

2. Materials and methods

2.1. Site description

This study was carried out in Jinotega, located in Northern Nicaragua, which has an estimated population of 41,134 inhabitants of which 11% are children under 5 years of age. The city is located at 1,074 meters above sea level where the temperature ranges from 18–32°C. The majority of the population is involved in agricultural activities related to the production of coffee. Secondary medical care for Gynecology-Obstetrics, General Surgery, Internal Medicine, Pediatrics, Neonatology, and Orthopedics was provided by Victoria Motta Hospital, Jinotega.

2.2. Study design

From April to August in 2010, a hospital-based study of sporadic acute diarrhea was performed. A total of 107 children of 5 years of age with acute diarrhea were enrolled in a longitudinal, prospective manner from either the emergency or pediatric room. After informed consent was acquired, epidemiological information was obtained for each case from the parents or guardians of the sick child. The Ethical Committee for Biometrics Research (registration no. 61) of the Faculty of Medical Sciences at UNAN-León approved this study.

2.3. RotaTeq immunization assessment

In October of 2006, the Nicaraguan Expanded Program of Immunization initiated a universal RV vaccination program with RotaTeq. RotaTeq is orally administered in a 3-dose regimen to children at 2, 4, and 6 months of age. The dates each child received vaccine doses were registered by an EPI nurse on the child's vaccination-card. The RotaTeq immunization data used in this study were collected from the children's vaccination-cards. A

child was considered “unvaccinated” if their vaccination card showed no recorded doses. RotaTeq immunization status was considered “unknown” if the child’s vaccination card was not available.

2.4. Clinical assessment

The clinical information for symptoms such as fever ($>38^{\circ}\text{C}$), nausea, vomiting, loss of appetite, abdominal cramps, abdominal distension (gas), and number of loose stools in the previous 24 h, dehydration status, and treatment plans were obtained by reviewing the information registered in clinical paper files. As proposed in the strategy for diarrhea management by the World Health Organization, all children involved in the study were clinically evaluated by pediatricians or general practitioners following the protocol for integrated management of childhood illness (IMCI). In brief, the IMCI protocol states that a child with diarrhea must be classified by dehydration status into one of the following categories: “severe-dehydration,” “some-dehydration,” and “without-dehydration”. Severely dehydrated children require immediate intravenous rehydration.

2.5. Sampling and preliminary analysis

Fecal specimens were collected in sterile containers 24 h after admission, and transported weekly at 4°C to the microbiology laboratory of UNAN-León. A 10% (wt/vol) suspension of stool material was prepared with phosphate-buffered saline ($\text{pH} = 7.2$), and two aliquots were frozen at -20°C for later testing of the samples for RV and other viruses.

2.6. Viral antigen detection

A direct enzyme immunoassay for detection of RV in fecal specimens, OXOID ProSpecT™ R240396, was used (Cambridge, UK), according to the manufacturer’s instructions. The results were visually read and confirmed by absorbance measurements. Astrovirus and adenovirus co-infections in RV-positive samples were evaluated using IDEIA K6042 Astrovirus (Dako Cytomation Ltd) and ProSpecT™ Adenovirus (OXOID Ltd) enzyme immunoassay kits. The procedures were carried out according to the manufacturer’s instructions, and the results were visually read and confirmed by absorbance measurements. Noroviruses were detected using the procedure described by (Nordgren et al., 2008).

2.7. RNA extraction

Viral RNA was extracted from stool suspensions using a Qiagen QIAmp viral RNA mini kit (Hilden, DE) according to the manufacturer’s instructions. A total of 60 μl of purified viral RNA was obtained and stored at -20°C until used in reverse transcription (RT) and polymerase chain reaction (PCR).

2.8. RT-PCR

RT was carried out as described previously (Bucardo et al., 2008). Briefly, 28 μl of purified RNA was mixed with 50 pmol of random hexadeoxynucleotides [pd(N)6] (GE Healthcare Life Sciences), and the mixture was denatured at 97°C for 5 min and quickly chilled on ice for 2 min, followed by the addition of one RT bead (Amersham Biosciences, UK) and RNase-free water to a final volume of 50 μl . RT reaction mixtures were incubated for 30 min at 42°C to produce cDNA.

2.9. G and P multiplex genotyping

The G and P genotypes of RVs recovered from stool samples were determined by PCR (Gouvea et al., 1990; Iturriza-Gomara et al., 2004). The generic and genotype-specific primers used for detecting VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12 were described previously (Gomara et al., 2001; Iturriza-Gomara et al., 2004; Samajdar et al.,

2006). Primers used for detecting VP4 genotypes P[4], P[6], P[8], and P[9] were also described previously (Gentsch et al., 1992; Iturriza-Gomara et al., 2004).

2.10. Genome sequencing

Superscript One-Step RT-PCR kits (Invitrogen) were used to generate cDNAs from RNAs recovered from stool samples. Reaction mixtures contained universal primer pairs specific to each of the RV genes (Matthijnssens et al., 2008). The cDNA products were resolved by electrophoresis on agarose gels and purified using a QIAquick PCR purification kit (Qiagen). The purified cDNAs were sequenced with an ABI Prism BigDye v3.1 terminator cycle sequencing kit and detected with an Applied Biosystems 3730 DNA Analyzer. The sequence files were assembled and analyzed using Sequencher 4.7 software (Gene Codes Corporation).

2.11. Phylogenetic trees, sequence analysis, and statistics

Maximum-likelihood phylogenetic trees were reconstructed using PhyML (Guindon and Gascuel, 2003) employing the Hasegawa-Kishino-Yano substitution model (HKY85) and gamma-distributed rate variation among sites. Bootstrap analysis was performed based on 1000 replicates and trees were visualized using Geneious v5.5.6 (<http://www.geneious.com/>). Multiple sequence alignments for VP4, VP7 and NSP2 were prepared using CLUSTALW (v1.83) within the MacVector 12.0 suite. Statistical analysis was performed using IBM SPSS version 19 software (Chicago, IL). P-values of <0.05 were considered statistically significant.

2.12. Nucleotide accession numbers

The Genbank accession numbers for the 12 Jinotega RV strains sequenced in this study are given in Table 1.

3. Results

3.1. Epidemiological profile of RV infections in children immunized with RotaTeq

RV was detected in 18 of 107 (17%) stool samples collected from diarrheic children that were 5 years of age (Table 2). The incidence of RV-infection in diarrheic children was similar in boys (16%) and girls (17%) and was greatest in children that were 2 – 5 years of age (24%) and least frequent in those 6 months (5%). Similar rates of infections were observed in children living in rural (18%) and urban (16%) areas. The incidence of RV infection was greatest in April (42%) and decreased markedly in subsequent months (May – August). The increase in RV activity in April was associated with a sharp increase in patient consultations for diarrhea at the hospital (date not shown). Norovirus and astrovirus coinfections were not detected in any RV-positive stool samples; an adenovirus co-infection was observed in one (data not shown).

3.2. Clinical profile of RV-positive children

RV-positive children presented with symptoms that included watery diarrhea (89%), vomiting (89%), fever of 38°C (72%), and 4 liquid stools in a 24 h period (83%) (Table 3). Of the 18 RV-positive children, 10 (56%) were classified as severely dehydrated, 7 (39%) as moderately dehydrated, and 1 (5%) as not dehydrated. Immediate intravenous rehydration was required for RV-positive children with severe dehydration (10/18) and children with moderate dehydration (2/18) that did not tolerate oral rehydration solution.

3.3. RV diarrhea in children immunized with RotaTeq

Of the 18 RV-positive children, 12 (66%) were known to have received at least two vaccine doses, 2 (11%) were unvaccinated, and the vaccine status of 4 (22%) was unknown (Table 4). Of the 12 children known to have been vaccinated, 8 had received the complete three dose series, while 4 has received only the first two doses (Table 5). The final vaccine dose had been administered to RV-positive children at least 1 month (32 – 744 days) prior to stool collection.

3.4. VP7 and VP4 genotypes

The genotypes of RVs causing diarrheal disease in vaccinated children was determined by PCR genotyping and/or sequencing. The analysis showed that of the 12 vaccinated children (Table 5), 11 were infected with G1P[8] viruses and 1 with a G3P[8] virus.

3.5. Genome constellations

Genome sequencing allowed genotype assignment of all eleven genes of the RVs causing diarrheal disease in vaccinated children (Table 5). Nine of the G1P[8] viruses (designated “Jinotega-G1/N1” strains) contained prototypic genogroup 1 genome constellations (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1). Unexpectedly, two other G1P[8] viruses (NIC9J and NIC25J; designated “Jinotega G1/N2” strains) contained the genome constellation G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1, indicating that they contained genotype 2 NSP2 genes. Nucleotide sequence comparisons revealed that the genotype 2 NSP2 genes of the Jinotega G1/N2 strains were identical to the bovine WC3 NSP2 genes of the RotaTeq viruses. Based on additional sequence analysis and inspection of phylogenetic trees (Fig. 1, NSP2 tree), the NSP2 gene was the only one of the eleven genes of the two Jinotega G1/N2 strains originating from the RotaTeq vaccine. Thus, the Jinotega G1/N2 strains appear to have been generated by reassortment of G1P[8] viruses with RotaTeq viruses. Notably, the children infected with the Jinotega G1/N2 strains had previously received 2 or 3 doses of the RotaTeq vaccine (Table 5).

Of RVs causing disease in vaccinated children, only one was not a G1P[8] virus (Table 5). Instead, this was a G3P[8] virus with the genogroup 1 genome constellation of G3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 (designated the “Jinotega-G3” strain).

3.6. Phylogenetic analysis

To further explore the genetic relationships of the 12 Jinotega strains, maximum likelihood phylogenetic trees were constructed for each viral gene (Fig. 1). Sequences clustering within a single distinguishable branch were used to identify subgenotype alleles for the non-VP4, VP7 genes. For ease of data analysis, the alleles were color-coded (yellow, blue, green) in the trees. The allele-based genome constellations of the Jinotega strains are presented in Fig. 2. The analysis showed that the allele composition of all 11 genes of 8 G1P[8] strains (yellow) was identical suggesting that these viruses represent the same strain. In contrast, the VP6 (blue) and NSP2 (blue, green) allele composition of the 3 G1P[8] strains NIC7J, NIC9J, and NIC25J differed from each other and from those of the other 8 G1P[8] strains. Thus, these three viruses are genetically distinct and differ in origin. Most surprisingly, although the Jinotega G1/N2 strains NIC9J and NIC25J both contained identical RotaTeq N2-genotype alleles, they contained phylogenetically distinct VP6 alleles (Fig. 1, VP6 tree). From this, we can conclude that the NIC9J and NIC25J strains, which contain identical vaccine-derived NSP2 genes, are genetically different from one another and therefore must have distinct evolutionary histories.

Several alleles detected in the single Jinotega G3P[8] isolate (e.g., VP1, VP2, VP3, NSP1, NSP3) were not present in any of the G1P[8] viruses (Fig. 1 and 2). This indicates that the

G3P[8] isolate is evolutionarily distant from the Jinotega G1P[8] viruses and appears not to have originated simply by genetic replacement (via reassortment) of the VP7 gene of one of the commonly circulating G1P[8] viruses with a G3 VP7 gene.

3.7. Antigenic epitopes of Jinotega RVs

Phylogenetic analysis indicates that the G1 VP7 genes of the Jinotega strains cluster within the lineage Ic branch while the RotaTeq G1 VP7 gene clusters within lineage III (Fig. 1, VP7 G1 tree). The Jinotega G1 VP7 proteins have an overall identity value of 93–94% with the RotaTeq G1 VP7 protein, and there are 5 or 6 amino acids on the surface-exposed face of the Jinotega VP7 proteins that differ from that of RotaTeq G1 VP7 (Fig. 3A). Three or four of the differences are located within the structurally defined 7-1a (S94N, E97E/G, N123S, R291K) antigenic domain and one each are within the 7-1b (T217M) and 7-2 (N147S) antigenic domains. The 7-1a, 7-1b, and 7-2 domains include antigenic regions A and D; C, E and F; and B, respectively (Fig. 3B) (Aoki et al., 2009). Analysis of sequences deposited in GenBank indicates that RVs with Jinotega-like G1 VP7 sequences (99% identity) have been identified in several countries over the last decade, including several which were described prior to the introduction of RV vaccines [e.g., India-2006 (Genbank ACZ04410), US-2005 (AEB800120), Australia-2004 (AEB794020)]. Thus, the Jinotega G1 VP7 proteins appear neither to be unique nor recently evolved.

The G3 VP7 gene of the single Jinotega G3P[8] strain (NIC64J) phylogenetically clusters within the same lineage branch (III) as the G3 VP7 gene of RotaTeq (Fig. 1, VP7 G3 tree). However, because there are more than 50 nucleotide differences between the VP7 genes of the Jinotega G3P[8] and RotaTeq G3 strains (data not shown), the Jinotega G3 VP7 gene is not likely to have arisen via reassortment with the vaccine strain. Of the 10 amino acids that differ between the VP7 proteins of the Jinotega G3P[8] and RotaTeq G3 strains, only four are present on the surface-exposed face of the protein (Fig. 3A). Three are located in the 7-1b (T213A, D238K, N242D) antigenic domain and one is in the 7-2 (M148L) domain. The amino acid sequences of the 7-1a domains of the NIC64J and RotaTeq G3 VP7 proteins are identical (Fig. 3B). G3P[8] viruses that have VP7 proteins almost identical in sequence (99% identity) to that of NIC64J have been isolated recently in many countries; these include China-2007 (Genbank AF260958), Japan-2007 (ADU87021), US-2008 (AEB80035), and Vietnam-2006 (ABJ90333). Hence, like the Jinotega G1 VP7 proteins, the Jinotega G3 VP7 protein appears not to be unique, but rather represents a form of the G3 protein that is broadly distributed throughout the world.

The VP4 proteins of the Jinotega G1P[8] and G3P[8] strains belong to lineage 3 (Zeller et al., 2012), are nearly identical (>99%) in sequence, and the residues that make up the antigenic domains of their VP8* (8-1 to 8-4) and VP5* (5-1 to 5-5) fragments are all the same. However, the Jinotega P[8] VP4 proteins are less similar to RotaTeq P[8] VP4 (lineage 2), with a sequence identity of 95–96% (Fig. 1, VP4 tree). Five residues of the antigenic domains of Jinotega P[8] VP4 differ from those of RotaTeq P[8] VP4: two in the 8-1 domain (G146>D, G196>D), one in the 8-3 domain (D113>N), and two in the 5-1 domain (S384>R, D386>H) (Fig. 4). In contrast, no differences were noted in the amino acid residues of several other antigenic domains (8-2, 8-4, 5-2, 5-3, 5-4, and 5-5) of the Jinotega and RotaTeq P[8] VP4 proteins. The Jinotega P[8] VP4 sequences are nearly identical (99%) to P[8] VP4 sequences reported for G1P[8], G3P[8], and G9P[8] viruses that were isolated at various locations in the US (Genbank AEH41315, ADK46486, AEG79760, AEB79762) and Australia (AEB79194) from 2004–2009, indicating that the Jinotega viruses do not have unusual P[8] VP4 proteins.

3.8. Amino acid analysis of VP6 and NSP2

VP6 subgroup (SG) specificity can be defined based on the identity of surface residues on VP6 trimers that comprise the recognition epitopes for SG-specific monoclonal antibodies. The typical residues of the SG-I epitope include A172 and A305 and those of the SG-II epitope include M172, N305, Q310, and Q315 (Greig et al., 2006; López et al., 1994). Analysis of the VP6 protein sequences of the Jinotega strains revealed that they all contained the latter set of amino acid residues, allowing the assignment of these viruses to SG-II (data not shown).

The sequences of the NSP2 proteins of the Jinotega G1/N2 strains are the same (100% identity) as those of the RotaTeq vaccine but share lower identity (88–89%) with those of the Jinotega G1 strains. None of the differences between the NSP2 proteins of the Jinotega G1 and G1/N2 strains include residues known to be involved in the protein's NTPase and RTPase activities (Fig. 5A) (Kumar et al., 2007; Vasquez-Del Carpio et al., 2006). Residues involved in the RNA-binding activity of NSP2 are also shared between Jinotega G1/N2 strains and some Jinotega G1 strains. In the infected cell, NSP2 self assembles into doughnut-shaped octamers formed by the stacking of two NSP2 tetramers (Fig. 5B) (Jiang et al., 2006; Taraporewala et al., 2006; Jayaram et al., 2002). Running tangentially across the face of the octamer are four deep grooves; these serve as RNA-binding sites and contain access points to the NTPase/RTPase catalytic sites. Sequence differences between the NSP2 proteins of the Jinotega G1 and G1/N2 strains are distributed over the entire surface of the octamer; none map to the grooves. Thus, the sequence differences may not have any impact on the function of NSP2, allowing the Jinotega G1/N2 strains to maintain viability and retain the potential to cause human disease despite their unusual genome constellations.

4. Discussion

In late April 2010, a sharp increase in patient consultations for diarrheal illness occurred at Victoria Motta, a hospital in Jinotega, Nicaragua. Samples collected from children with severe diarrhea revealed that RV was a common cause of infection and that most of the RV-positive children (12/18) had received at least two doses of RotaTeq vaccine (Table 5). In the months of May – August 2010 the frequency of RV-related disease decreased. Analysis of twelve vaccinated children hospitalized with RV-positive diarrheal disease showed that eleven were infected with G1P[8] viruses and one with a G3P[8] virus. Our study is not the first to identify G1 RVs as causative agents of diarrheal disease in children vaccinated with RotaTeq. Indeed, similar observations were made during clinical trials with RotaTeq carried out in developed countries (US and Finland) and developing countries (Bangladesh and Vietnam) (Vesikari et al., 2006; Zaman et al., 2010).

Given that RotaTeq can induce protective immunity against RVs with G1, G2, G3, G4 and P[8] specificities, the finding that the RotaTeq vaccine failed to protect a number of Jinotega children against G1P[8]/G3P[8] RV disease is important, as it suggests that factors other than vaccine composition might impact vaccine efficacy. Due to the fact that children in Nicaragua are frequently exposed to a variety of enteric pathogens, one possibility was that diarrheal disease in the vaccinated Jinotega children was not due to RV infection, but due to a secondary infection. However, assay of the children's stool samples for adenovirus, astrovirus, norovirus, and enterotoxigenic *Escherichia coli* (ETEC) identified only 3 of the 18 children as positive for other agents (*vis-à-vis*, adenovirus or ETEC). Thus, RV is the likely cause of most, if not all, of the children's diarrheal illness. Another possibility for the RV-positive test results in the Jinotega children was that they were shedding vaccine viruses because of recent immunization with RotaTeq. Indeed, a recent report has indicated that children receiving the first dose of RotaTeq may excrete vaccine virus for up to 9 days (Yen et al., 2011a). However, genome sequencing of the RVs collected from the Jinotega children

excluded this possibility, as all eleven genes of the viruses differed markedly from those of RotaTeq viruses, with the exception of the NSP2 N2 gene of Jinotega G1/N2 strains.

A number of host factors have been suggested to impact whether RV vaccines can induce an adequate protective response in the child and whether the vaccinated child can avoid diarrheal disease when subsequently exposed to the virus. These factors include breastfeeding and the presence or absence of immune and non-immune components in breast milk, and the nutrition status of the child including possible vitamin deficiencies (Vesikari et al., 2012; Chan et al., 2011; Wobudeya et al., 2011; Johansson et al., 2008; Moon et al., 2010; Patel et al., 2009b; Goveia et al., 2008). Unfortunately, we lack sufficient information for the vaccinated Jinotega children to evaluate how these factors may have contributed to their illness.

Sequencing of the G1P[8] and G3P[8] RVs recovered from vaccinated Jinotega children with diarrheal disease revealed that, by-in-large, the viruses had genome constellations typical of Wa-like genogroup 1 viruses (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1). The two exceptions were the Jinotega G1/N2 strains; these had genome constellations that indicated the presence of a bovine genotype N2 NSP2 gene instead of the expected genotype N1 NSP2 gene. Surprisingly, the sequence of the N2 NSP2 gene was identical to that found in RotaTeq vaccine strains. Thus, the Jinotega G1/N2 strains likely originated by co-infection and reassortment of a human G1P[8] virus with a RotaTeq strain in a vaccinee. Since we found no evidence of other RotaTeq genes in the stool samples of the children from which Jinotega G1/N2 strains were recovered, it is not likely that the G1P[8] x RotaTeq reassortment event occurred in these children. Rather, the children were most likely infected with these reassortants, which indicates that these G1/N2 strains are viable, can spread, and can cause disease. Similarly, Maan et al (2010) have reported that live attenuated vaccine against bluetongue virus, another segmented dsRNA virus of the *Reoviridae*, can reassort with circulating wildtype strains in vivo to form viable reassortants capable of spreading and causing disease.

In our analysis, we contrasted the amino sequences of the G1 and G3 VP7 and P[8] VP4 proteins of the Jinotega virus strains with those of the RotaTeq strains, looking for differences in antigenic epitopes that might explain the failure of the vaccine to protect some Jinotega children against RV disease. The results indicated that 5 or 6 surface-exposed residues within the antigenic domains of the VP7 protein differed between the Jinotega and RotaTeq G1 viruses (Fig. 3). Four such residues differed between the Jinotega and RotaTeq G3 viruses. Despite these differences, large expanses of the immunodominant 7-1a/7-1b antigenic domains of the VP7 proteins were the same for the Jinotega G1 and G3 and RotaTeq G1 and G3 viruses, respectively (Fig. 3B). Comparison of the P[8] VP4 proteins of the Jinotega and RotaTeq viruses indicated that only two (8-1, 8-3) of the four antigenic epitopes of the VP8* fragment and one (5-1) of the five antigenic epitopes of the VP5* fragment contained differences in surface-exposed residues (Fig. 4). Thus, like the VP7 protein, large expanses of the P[8] VP4 protein including regions that contain antigenic epitopes, were the same for the Jinotega and RotaTeq P[8] viruses. From this analysis, one can predict that the RotaTeq vaccine can induce homotypic protective responses in children capable of preventing diarrheal disease by the Jinotega G1P[8] and G3P[8] virus strains. As a result, factors other than vaccine composition are likely the cause of the vaccine failures observed for the Jinotega children. Thus, reformulation of existing vaccines to include additional G or P genotypes may not prove particularly useful in achieving greater levels of protection in vaccines of developing countries. Our analysis, albeit limited to a relative few virus isolates, suggests that increased genetic diversity of circulating RVs does not explain the higher vaccine failure rate in at least some developing countries. The isolation of RVs with vaccine-derived genes from children in countries using RotaTeq suggests that the

widespread use of vaccine strains will have an impact on the genetic composition and evolution of circulating viruses that are associated with human disease. The extent to which this occurs will require RV surveillance programs that include full genome sequencing efforts.

Acknowledgments

We would like to express our appreciation to Angelica Castro and Cristel Escoto for help in collecting samples, Dra. Gioconda Ramirez for providing diarrhea surveillance data, and Dr. Samuel Vilchez for assistance with ETEC PCR analysis. This study was supported in part by NETROPICA (grant 05-N-2010) and a post-doctoral small research grant from UNAN-León and SIDA. JTP and CMR were supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (USA).

References

- 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; 324:1444–1447. [PubMed: 19520960]
- Bucardo F, Nordgren J, Carlsson B, Paniagua M, Lindgren PE, Espinoza F, Svensson L. Pediatric norovirus diarrhea in Nicaragua. *J Clin Microbiol*. 2008; 46:2573–2580. [PubMed: 18562593]
- Carvalho-Costa FA, Volotao Ede M, de Assis RM, Fialho AM, de Andrade Jda S, Rocha LN, Tort LF, da Silva MF, Gomez MM, de Souza PM, Leite JP. Laboratory-based rotavirus surveillance during the introduction of a vaccination program, Brazil, 2005–2009. *Pediatr Infect Dis J*. 2011; 30:S35–41. [PubMed: 21048523]
- Chan J, Nirwati H, Triasih R, Bogdanovic-Sakran N, Soenarto Y, Hakimi M, Duke T, Buttery JP, Bines JE, Bishop RF, Kirkwood CD, Danchin MD. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine*. 2011; 29:1242–1247. [PubMed: 21147127]
- Clark HF, Offit PA, Plotkin SA, Heaton PM. The new pentavalent rotavirus vaccine composed of bovine (strain WC3) -human rotavirus reassortants. *Pediatr Infect Dis J*. 2006; 25:577–583. [PubMed: 16804425]
- 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; 201:363–369. [PubMed: 20047501]
- Coulson B. VP4 and VP7 typing using monoclonal antibodies. *Archives of Virology Supplement*. 1996; 12:113–118.
- Coulson BS, Kirkwood C. Relation of VP7 amino acid sequence to monoclonal antibody neutralization of rotavirus and rotavirus monotype. *J Virol*. 1991; 65:5968–5974. [PubMed: 1656083]
- Donker NC, Foley M, Tamvakis DC, Bishop R, Kirkwood CD. Identification of an antibody-binding epitope on the rotavirus A non-structural protein NSP2 using phage display analysis. *J Gen Virol*. 2011; 92:2374–2382. [PubMed: 21697352]
- 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; 21:885–897. [PubMed: 11867517]
- Dyall-Smith ML, Lazdins I, Tregear GW, Holmes IH. Location of the major antigenic sites involved in rotavirus serotype-specific neutralization. *Proc Natl Acad Sci U S A*. 1986; 83:3465–3468. [PubMed: 2422651]
- Estes, MK.; Kapikian, A. Rotaviruses. In: Knipe, DM.; Griffin, PHDE.; Lamb, RA.; Martin, M.; Roizman, B.; Straus, SE., editors. *Fields Virology*. Kluwer Health/Lippincott, Williams and Wilkins; Philadelphia: 2007. p. 1917–1975.
- 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; 30:1365–1373. [PubMed: 1320625]

- Gomara MI, Cubitt D, Desselberger U, Gray J. Amino acid substitution within the VP7 protein of G2 rotavirus strains associated with failure to serotype. *J Clin Microbiol.* 2001; 39:3796–3798. [PubMed: 11574622]
- 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; 28:276–282. [PubMed: 2155916]
- Goveia MG, DiNubile MJ, Dallas MJ, Heaton PM, Kuter BJ. REST Study Team. Efficacy of pentavalent human-bovine (WC3) reassortant rotavirus vaccine based on breastfeeding frequency. *Pediatr Infect Dis J.* 2008; 27:656–658. [PubMed: 18520448]
- Greig SL, Berriman JA, O'Brien JA, Taylor JA, Bellamy AR, Yeager MJ, Mitra AK. Structural determinants of rotavirus subgroup specificity mapped by cryoelectron microscopy. *J Mol Biol.* 2006; 356:209–221. [PubMed: 16359700]
- Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol.* 2003; 52:696–704. [PubMed: 14530136]
- Gurgel RQ, Cuevas LE, Vieira SC, Barros VC, Fontes PB, Salustino EF, Nakagomi O, Nakagomi T, Dove W, Cunliffe N, Hart CA. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. *Emerg Infect Dis.* 2007; 13:1571–1573. [PubMed: 18258011]
- Heaton PM, Ciarlet M. Vaccines: the pentavalent rotavirus vaccine: discovery to licensure and beyond. *Clin Infect Dis.* 2007; 45:1618–1624. [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; 82:11106–11116. [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; 332:177–188. [PubMed: 15661150]
- Hoshino Y, Kapikian AZ. Classification of rotavirus VP4 and VP7 serotypes. *Arch Virol Suppl.* 1996; 12:99–111. [PubMed: 9015107]
- 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, Poljsak-Prijatelj M, Pothier P, Ruggeri FM, Sanchez-Fauquier A, Steyer A, Sitaraviciute-Ivaskeviciene I, Syriopoulou V, Tran AN, Usonis V, VANR M, DER A, Gray J. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. *Epidemiol Infect.* 2011; 139:895–909. [PubMed: 20707941]
- Iturriza-Gomara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *J Clin Virol.* 2004; 31:259–265. [PubMed: 15494266]
- Jayaram H, Taraporewala Z, Patton JT, Prasad BV. Rotavirus protein involved in genome replication and packaging exhibits a HIT-like fold. *Nature.* 2002; 417:311–315. [PubMed: 12015608]
- Jiang X, Jayaram H, Kumar M, Ludtke SJ, Estes MK, Prasad BV. Cryoelectron microscopy structures of rotavirus NSP2-NSP5 and NSP2-RNA complexes: implications for genome replication. *J Virol.* 2006; 80:10829–10835. [PubMed: 16928740]
- Johansson E, Istrate C, Charpilienne A, Cohen J, Hinkula J, Poncet D, Svensson L, Johansen K. Amount of maternal rotavirus-specific antibodies influence the outcome of rotavirus vaccination of newborn mice with virus-like particles. *Vaccine.* 2008; 26:778–785. [PubMed: 18191310]
- Kumar M, Jayaram H, Vasquez-Del Carpio R, Jiang X, Taraporewala ZF, Jacobson RH, Patton JT, Prasad BV. Crystallographic and biochemical analysis of rotavirus NSP2 with nucleotides reveals a nucleoside diphosphate kinase-like activity. *J Virol.* 2007; 81:12272–12284. [PubMed: 17804496]
- López S, Espinosa R, Greenberg HB, Arias CF. Mapping the subgroup epitopes of rotavirus protein VP6. *Virology.* 1994; 204:153–162. [PubMed: 7522369]
- Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, Ngwira B, Victor JC, Gillard PH, Cheuvart BB, Han HH, Neuzil KM. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med.* 2010; 362:289–298. [PubMed: 20107214]

- Maan S, Maan NS, van Rijn PA, van Gennip RG, Sanders A, Wright IM, Batten C, Hoffmann B, Eschbaumer M, Oura CA, Potgieter AC, Nomikou K, Mertens PP. Full genome characterisation of bluetongue virus serotype 6 from the Netherlands 2008 and comparison to other field and vaccine strains. *PLoS One*. 2010; 5:e10323. [PubMed: 20428242]
- 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*. 2008; 82:3204–3219. [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, John R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreno V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol*. 2011; 156:1397–1413. [PubMed: 21597953]
- Matthijnssens J, Joelsson DB, Warakowski 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, RotaTaq. *Virology*. 2010; 403:111–127. [PubMed: 20451234]
- 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:e1000634. [PubMed: 19851457]
- Moon SS, Wang Y, Shane AL, Nguyen T, Ray P, Dennehy P, Baek LJ, Parashar U, Glass RI, Jiang B. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J*. 2010; 29:919–923. [PubMed: 20442687]
- Nakagomi T, Cuevas LE, Gurgel RG, Elrokhsi SH, Belkhir YA, Abugalia M, Dove W, Montenegro FM, Correia JB, Nakagomi O, Cunliffe NA, Hart CA. Apparent extinction of non-G2 rotavirus strains from circulation in Recife, Brazil, after the introduction of rotavirus vaccine. *Arch Virol*. 2008; 153:591–593. [PubMed: 18175037]
- Nordgren J, Bucardo F, Dienus O, Svensson L, Lindgren PE. Novel light-upon-extension real-time PCR assays for detection and quantification of genogroup I and II noroviruses in clinical specimens. *J Clin Microbiol*. 2008; 46:164–170. [PubMed: 17959761]
- 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; 9:565–572. [PubMed: 12737740]
- Patel M, Pedreira C, De Oliveira LH, Tate J, Orozco M, Mercado J, Gonzalez A, Malespin O, Amador JJ, Umana J, Balmaseda A, Perez MC, Gentsch J, Kerin T, Hull J, Mijatovic S, Andrus J, Parashar U. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA*. 2009a; 301:2243–2251. [PubMed: 19491186]
- Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: how well will they work where they are needed most? *The J Infect Dis*. 2009b; 200(Suppl 1):S39–48.
- 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; 36:183–188. [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; 15:29–56. [PubMed: 15484186]
- Taraporewala ZF, Jiang X, Vasquez-Del Carpio R, Jayaram H, Prasad BV, Patton JT. Structure-function analysis of rotavirus NSP2 octamer by using a novel complementation system. *J Virol*. 2006; 80:7984–7994. [PubMed: 16873255]
- 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.* 2012; 12:136–141. [PubMed: 22030330]
- Vasquez-Del Carpio R, Gonzalez-Nilo FD, Riadi G, Taraporewala ZF, Patton JT. Histidine triad-like motif of the rotavirus NSP2 octamer mediates both RTPase and NTPase activities. *J Mol Biol.* 2006; 362:539–554. [PubMed: 16934294]
- Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, Meurice F, Han HH, Damaso S, Bouckennooghe A. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet.* 2007; 370:1757–1763. [PubMed: 18037080]
- Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB, Shinefield HR, Christie CD, Ylitalo S, Itzler RF, Coia ML, Onorato MT, Adeyi BA, Marshall GS, Gothefors L, Campens D, Karvonen A, Watt JP, O'Brien KL, DiNubile MJ, Clark HF, Boslego JW, Offit PA, Heaton PM. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med.* 2006; 354:23–33. [PubMed: 16394299]
- Vesikari T, Prymula R, Schuster V, Tejedor JC, Cohen R, Bouckennooghe A, Damaso S, Han HH. Efficacy and immunogenicity of live-attenuated human rotavirus vaccine in breast-fed and formula-fed European infants. *Pediatr Infect Dis J.* 2012 Jan 5. Epub ahead of print.
- WHO. Rotavirus vaccines: an update. WHO, 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.
- Wobudeya E, Bachou H, Karamagi CK, Kalyango JN, Mutebi E, Wamani H. Breastfeeding and the risk of rotavirus diarrhea in hospitalized infants in Uganda: a matched case control study. *BMC Pediatr.* 2011; 11:17. [PubMed: 21329521]
- Yen C, Jakob K, Esona MD, Peckham X, Rausch J, Hull JJ, Whittier S, Gentsch JR, LaRussa P. Detection of fecal shedding of rotavirus vaccine in infants following their first dose of pentavalent rotavirus vaccine. *Vaccine.* 2011a; 29:4151–4155. [PubMed: 21477676]
- 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
- 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, Schodel 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; 376:615–623. [PubMed: 20692031]
- Zeller M, Patton JT, Heylen E, DeCoster S, Ciarlet M, Van Ranst M, Matthijssens 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.* 2012; 50:966–976. [PubMed: 22189107]

Highlights

- The rotavirus vaccine RotaTeqTM is widely used in Nicaragua
- Many children will contract rotavirus diarrheal illness despite prior vaccination
- Common global rotavirus strains are the cause of disease in vaccinated children
- Some strains recovered from children have genome segments that are vaccine derived
- Reassortment occurs between circulating rotaviruses and vaccine viruses
- Human-vaccine reassortant viruses are viable and can cause disease

Figure 1.

Genetic relationships among the genes of Jinotega RVs. Maximum-likelihood trees (midpoint rooted) were constructed for each gene using the ORF nucleotide sequences of the Jinotega isolates, the RotaTeq (W179-9) strain (boxed in red), and the necessary reference strains required to define lineages or genotypes (labeled to the right of each tree). Horizontal branch lengths are drawn to scale (nucleotide substitutions per base), and bootstrap values of above 70 (%) are shown for key nodes. Genome segments of Jinotega strains within different branches were used to define distinct alleles; the alleles are color-coded (yellow, blue, or green).

Figure 2.

Allele-based genome constellations of Jinotega RVs. The schematic illustrates the color-coding of each gene as determined by the phylogenetic trees shown in Figure 1. The strain name and G/P-specificity are shown on the left and the protein encoded by the genes is presented at the top. The G1P[8] strains (NIC9J and NIC25J) boxed in red contain genotype N2 NSP2 genes (green) that have sequences identical to those of the RotaTeq vaccine.

Figure 3.

Comparison of the VP7 antigenic epitopes of Jinotega and RotaTeg RVs. (A) Surface rendering of RV VP7 trimer (PDB 3FMG) from the perspective of the virion exterior, with residues color-coded to identify the structure-based antigenic domains 7-1a (red), 7-1b (salmon), and 7-2 (plum) (McDonald et al., 2009). Surface-exposed residues that differ between the Jinotega G1 (left) or G3 (right) VP7 proteins and the RotqTeg G1 or G3 proteins, respectively, are color-coded in cyan blue and labeled. (B) Amino acid alignment of VP7 proteins, with antigenic epitopes (A-F) and variable regions shaded in gray (Dyall-Smith et al., 1986; Matthijssens et al., 2010). Colored arrow heads correspond to residues shown in panel A that differ between the Jinotega and RotaTeg vaccine strains. Black arrow identifies the cleavage site in the VP7 glycoprotein used in removing the N-terminal signal sequence.

		VP8*																				VP5*																		
		8-1										8-2				8-3						8-4					5-1					5-2	5-3	5-4	5-5					
Lineage		100	145	147	149	187	189	191	192	193	194	195	179	182	113	114	115	116	125	131	132	133	135	86	87	88	89	90	383	385	387	392	393	397	439	440	433	458	428	305
RotaTeq (G6P[8])	2	D	S	S	N	S	N	A	N	L	N	D	E	R	N	P	V	D	N	R	N	D	D	S	N	T	N	G	R	H	S	A	W	N	L	R	E	N	S	L
NIC7J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC9J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC18J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC22J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC24J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC25J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC26J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC28J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC41J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC45J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC72J (G1P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L
NIC64J (G3P[8])	3	D	G	S	N	S	N	A	N	L	N	G	E	R	D	P	V	D	N	R	N	D	D	S	N	T	N	G	S	D	S	A	W	N	L	R	E	N	S	L

Figure 4.

Differences in the P[8] VP4 antigenic epitopes of RotaTeq and Jinotega RVs. Antigenic residues are resolved into four antigenic epitopes in VP8* and five antigenic epitopes in VP5*; the amino acid residues comprising the epitopes are identified across the top. Virus strains, their G/P-type specificity, and the lineage of their VP4 component are identified to the left. Residues that differ between the vaccine strain and the Jinotega strains have been boxed.

Figure 5. Comparison of the NSP2 sequences of Jinotega and RotaTeq RVs. (a) Amino acid alignment of NSP2 proteins, with residues involved in RNA-binding activity (blue arrowheads) and in NTP/RTPase hydrolysis (red arrowheads) identified. The recognition site for an NSP2 antibody that reduces virus replication is shaded in gray (Donker et al., 2011). Jinotega (NIC) virus strains and the RotaTeq virus strain WI79-9 are identified on the left, along with the NSP2 genotype (N1 or N2). (B). Surface rendering of the NSP2 octamer (PDB ID: 278F) shown in top and side views, with the individual monomers differentially colored (pink, yellow, green, blue). Surface-exposed residues that differ between the NSP2 proteins of the NIC-26J, -22J, -18J, -28J, -41J, -45J, and -72J strains and WI79-9 strains have been color-coded in cyan. One of the RNA-binding grooves of the octamer is boxed in the side view (right). The arrows point to access points along the groove to the NTPase/RTPase catalytic sites.

Table 1

Full strain names and accession numbers for the RV strains sequenced in this study from Nicaragua

Full strain name	Genbank accession numbers of the genome segment and its corresponding protein										
	9 (VP7)	4 (VP4)	6 (VP6)	1 (VP1)	2 (VP2)	3 (VP3)	5 (NSP1)	8 (NSP2)	7 (NSP3)	10 (NSP4)	11 (NSP5/6)
RVA/Human/NCA/7J/2010/G1P[8]	JN129112	JN129084	JN129098	JN129042	JN129056	JN129070	JN128972	JN128986	JN129000	JN129014	JN129028
RVA/Human/NCA/9J/2010/G1P[8]	JN129113	JN129085	JN129099	JN129043	JN129057	JN129071	JN128973	JN128987	JN129001	JN129015	JN129029
RVA/Human/NCA/18J/2010/G1P[8]	JN129114	JN129086	JN129100	JN129044	JN129058	JN129072	JN128974	JN128988	JN129002	JN129016	JN129030
RVA/Human/NCA/22J/2010/G1P[8]	JN129115	JN129087	JN129101	JN129045	JN129059	JN129073	JN128975	JN128989	JN129003	JN129017	JN129031
RVA/Human/NCA/24J/2010/G1P[8]	JN129116	JN129088	JN129102	JN129046	JN129060	JN129074	JN128976	JN128990	JN129004	JN129018	JN129032
RVA/Human/NCA/25J/2010/G1P[8]	JN129117	JN129089	JN129103	JN129047	JN129061	JN129075	JN128977	JN128991	JN129005	JN129019	JN129033
RVA/Human/NCA/26J/2010/G1P[8]	JN129118	JN129090	JN129104	JN129048	JN129062	JN129076	JN128978	JN128992	JN129006	JN129020	JN129034
RVA/Human/NCA/28J/2010/G1P[8]	JN129119	JN129091	JN129105	JN129049	JN129063	JN129077	JN128979	JN128993	JN129007	JN129021	JN129035
RVA/Human/NCA/41J/2010/G1P[8]	JN129120	JN129092	JN129106	JN129050	JN129064	JN129078	JN128980	JN128994	JN129008	JN129022	JN129036
RVA/Human/NCA/45J/2010/G1P[8]	JN129121	JN129093	JN129107	JN129051	JN129065	JN129079	JN128981	JN128995	JN129009	JN129023	JN129037
RVA/Human/NCA/64J/2010/G3P[8]	JN129122	JN129094	JN129108	JN129052	JN129066	JN129080	JN128982	JN128996	JN129010	JN129024	JN129038
RVA/Human/NCA/72J/2010/G1P[8]	JN129123	JN129095	JN129109	JN129053	JN129067	JN129081	JN128983	JN128997	JN129011	JN129025	JN129039

Table 2

Profile of diarrheic children in Jinotega, Nicaragua

	No. (%) of diarrheic children, n = 107	No. (%) of children with RV-diarrhea, n = 18
Gender		
male	61 (57)	10 (56)
female	46 (43)	8 (44)
Age (months)		
<= 6	19 (18)	1 (6)
7–12	32 (30)	6 (33)
13–24	35 (33)	6 (33)
25–60	21 (20)	5 (28)
Living		
rural	56 (52)	10 (56)
urban	51 (48)	8 (44)
Breastfeeding		
yes	65 (61)	8 (44)
no	38 (36)	8 (44)
unknown	4 (4)	2 (11)
Collection month		
April	31 (30)	13 (72)
May	26 (24)	2 (11)
June	16 (15)	2 (11)
July	29 (27)	1 (6)
August	5 (5)	0 (0)

Table 3

Clinical features of RV-infected children in Jinotega

	No. (%) of RV diarrhea, n = 18	No. (%) of non- RV diarrhea, n = 89
Vomiting	16 (89)	61 (68)
Fever 38°C	13 (72)	73 (82)
Appetite loss	8 (36)	45 (51)
Abdominal distension (gas)	5 (28)	10 (11)
Abdominal cramp	4 (22)	20 (22)
Loose stools in 24 hr		
> 6	8 (44)	38 (43)
4 – 6	7 (39)	39 (44)
1 – 3	3 (17)	12 (13)
Stools		
watery	16 (89)	73 (82)
loose	2(11)	16 (18)
Dehydration status		
severe	10 (56)	15 (17)
moderate	7 (39)	67 (75)
none	1 (5)	7 (8)

Table 4

RotaTeq vaccination status of children with acute diarrhea

RotaTeq doses	No. (%) of diarrheic children, n = 107	No. (%) of children with RV-diarrhea, n = 18
0	7 (7)	2 (11)
1	6 (6)	0 (0)
2	24 (22)	4 (22)
3	58 (54)	8 (44)
unknown	12 (11)	4 (22)

Epidemiological and clinical characteristics of RV-positive children and genome constellations of RV strains isolated from stool

Table 5

Strain	Gender ^a	Age (months) ^b	Dehydration status	RotaTeq doses (dates)			Genome constellation
				1st	2nd	3rd	
3J	F	13	C	-	-	-	
7J	F	26	A	30.04.08	27.06.08	-	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
8J	M	47	B	-	-	-	
9J	M	9	C	21.09.09	17.11.09	14.01.10	G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1
18J	M	8	A	09.10.09	10.12.09	10.02.10	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
19J	F	25	B	-	-	-	
21J	M	40	B	-	-	-	
22J	F	10	C	13.08.09	13.10.09	11.02.10	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
24J	M	5	C	19.01.10	19.03.10	-	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
25J	M	6	C	19.12.09	20.02.10	-	G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1
26J	F	7	C	24.11.09	21.01.10	-	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
27J	F	15	C	-	-	-	
28J	M	22	B	10.10.08	16.12.08	03.02.09	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
41J	M	31	C	19.12.07	22.02.08	19.04.08	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
45J	M	11	C	28.08.09	14.10.09	07.01.10	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
64J	F	22	C	14.10.08	17.12.08	24.02.09	G3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
72J	F	19	C	22.01.09	26.04.09	25.08.09	G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1
79J	M	15	C	-	-	-	

^aF: Female, M: Male

^bAge stool sample collected

^cDehydration: none (A), moderate (B), severe (C)