Rotavirus Group A Genotypes Detected through Diarrheal Disease Surveillance in Haiti, 2012

Mathew D. Esona, Josiane Buteau, Mentor Ali Ber Lucien, Gerard A. Joseph, Eyal Leshem, Jacques Boncy, Mark A. Katz, Michael D. Bowen,* and S. Arunmozhi Balajee

Centers for Disease Control and Prevention, Atlanta, Georgia; Laboratoire National de Santé Publique (National Public Health Laboratory), Port-au-Prince, Haiti

Abstract. Samples collected in 2012 through diarrheal disease surveillance in Haiti were tested for rotavirus by enzyme immunoassay and real time RT-PCR and positive samples were genotyped. The predominant genotypes were G1P[8] (29% prevalence) and G9P[8] (21%). The observed genotype prevalence was similar to that reported previously for other Caribbean countries.

Group A rotavirus (RVA) is the primary cause of acute gastroenteritis in children < 5 years of age worldwide. An estimated 453,000 children aged < 5 years die of RVA diarrhea each year, with > 85% of these deaths occurring in low-income countries.^{1,2} In Latin America and the Caribbean, RVA-associated gastroenteritis is responsible for an estimated 15,000 deaths, 75,000 hospitalizations, and 2 million clinic visits annually among children < 5 years of age.³ In Haiti alone, RVA causes an estimated 2,234 deaths annually among children < 5 years.⁴

After two safe and effective RVA vaccines became available, the World Health Organization (WHO) in 2007 recommended inclusion of RVA vaccine in the immunization programs of Europe and the Americas, and in 2009 expanded the recommendation to all infants < 32 weeks of age worldwide.⁵ Most Global Accelerated Vaccine Initiative (GAVI)-eligible countries in the Latin America and Caribbean have introduced RVA vaccines in their national immunization programs.³ In 2014, Haiti became the 14th GAVI-eligible, and last of five GAVI-eligible countries in the Latin American and Caribbean region, to introduce RVA vaccines in its national immunization program (PATH; www.path.org). Recent reports from these countries show significant declines in hospitalizations and deaths due to RVA and that these vaccines are safe and effective against severe RVA disease as well as a cost-effective intervention.^{3,6–8}

The high estimated burden of RVA disease in Haitian children, together with the effectiveness of RVA vaccines to prevent childhood deaths and hospitalizations, accentuates the need for Haiti's introduction of RVA vaccines to save children's lives. Baseline data on the genotypes of circulating RVA strains in Haiti do not exist currently. We report results of molecular characterization of RVA strains collected as part of a laboratory-based surveillance for diarrheal diseases.

Beginning in April 2012, four geographically diverse hospitals participated in laboratory-enhanced surveillance for diarrheal diseases in Haiti, as has been described previously and continues to date.⁹ Each hospital collected and transported whole stool specimens from hospitalized patients with acute diarrhea (maximum 15 specimens per week) to the National Public Health Laboratory (NPHL). For the purposes of the surveillance, acute diarrhea was defined as three or more episodes of acute watery diarrhea within 24 hours, with onset of symptoms within the past 7 days. Specimens received at the

NPHL were tested for RVA using Premier™ Rotaclone® (Meridian Diagnostics, Cincinnati, OH) enzyme immunoassay (EIA).⁹ Of the 392 specimens received at NPHL in April–June 2012, 200 were available for further analysis and shipped to the Rotavirus Surveillance Laboratory, U.S. Centers for Disease Control and Prevention (CDC) for further characterization. Of the 200 specimens, 38 were known to be from children < 5 years of age.

At the CDC, ribonucleic acid (RNA) was extracted from the 200 samples and tested for RVA using a NSP3 real-time reverse transcription polymerase chain reaction (qRT-PCR).¹⁰ Using the same RNA extracts, VP7 and VP4 genotyping was attempted on all RVA-positive samples using previously described protocols.¹¹

Overall, 5/200 (2.5%) specimens were positive by RotaClone EIA, and 14 (7%) specimens, including the five RotaClone-positive specimens, were positive by the qRT-PCR assay. These results corroborate the generally accepted concept that molecular techniques are more sensitive than immunoassays. The low prevalence of RVA in the present study may be attributed, in part, to two factors: the low percentage of children enrolled in this study (only 19% of samples were known to be from children < 5 years of age) and the fact that most samples were collected during the rainy season. The typical peak season for RVA disease in tropical countries is during the dry season (which is typically December to March in Haiti).¹² During a full year of RVA sentinel surveillance in Haiti,⁹ among patients with acute watery diarrhea, RVA was found in 13.5% of specimens from children < 5 years, 3.9% from children 5–18 years, and < 1% from persons > 18 years of age.

Out of the 14 qRT-PCR-positive samples, partial or complete G and P genotype could be assigned for 13 samples; two samples were G and P non-typeable (Table 1). G1P[8] was detected in 4/14 (28.6%) specimens followed by G9P[8] which was detected in 3/14 samples (21.4%). We also detected single cases of G2P[8], G12P[6], G12P[8], G2P[NT], and G1P[NT] (NT = non-typeable) (Table 1). Studies based on the molecular characterization of RVA into G and P genotypes in the Caribbean are scarce. However, similar to our findings in Haiti, the Dominican Republic,^{13,14} St. Vincent,¹⁵ and Cuba¹⁶ have all reported G1P[8] as the most common strain in circulation, followed by G9P[8]. The detection of genotype G12 for the first time in Haiti further confirms the emergence of this genotype globally.¹⁷

One important limitation of this study was that the number of samples tested and genotyped was small. Given that vaccination against RVA has begun in Haiti, it now may be difficult

*Address correspondence to Michael D. Bowen, Division of Viral Diseases, NCIRD, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30333. E-mail: mkb6@cdc.gov

TABLE 1
Rotavirus genotyping results for real-time RT-PCR positive samples

Sample no.	Sampling date	Rotavirus EIA result	Approximate age	Rotavirus result real-time RT-PCR result	Genotype
1	24/04/2012	Not tested	31 years	Positive	G1P[8]
2	24/04/2012	Positive	20 months	Positive	G9P[8]
3	3/05/2012	Negative	26 years	Positive	G1P[8]
4	3/05/2012	Negative	9 years	Positive	G2P[NT]
5	26/04/2012	Negative	35 years	Positive	GNT[NT]
6	30/04/2012	Positive	20 months	Positive	G1P[8]
7	30/04/2012	Negative	30 years	Positive	G12P[8]
8	27/04/2012	Positive	6 years	Positive	G1P[8]
9	8/05/2012	Negative	20 years	Positive	G2P[8]
10	9/05/2012	Positive	5 months	Positive	GNT[NT]
11	21/05/2012	Not tested	48 years	Positive	G12P[6]
12	Unknown	Negative	30 years	Positive	G1P[NT]
13	Unknown	Negative	Unknown	Positive	G9P[8]
14	Unknown	Positive	20 years	Positive	G9P[8]

NT = non-typeable; RT-PCR = reverse transcription polymerase chain reaction.

to obtain a complete picture of RVA strain types in Haiti pre-vaccine introduction unless one has access to an archived sample collection. In the early stages of vaccine introduction when vaccine coverage is low, more extensive studies may still be useful to assess the impact of the vaccine on genotype distribution.

Received June 30, 2014. Accepted for publication March 29, 2015.

Published online May 11, 2015.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the CDC or the U.S. Department of Health and Human Services.

Authors' addresses: Mathew D. Esona, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: mdi4@cdc.gov. Josiane Buteau, Gerard A. Joseph, and Jacques Boney, Laboratoire National de Santé Publique, LNSP, Port-au-Prince, Haiti, E-mails: jolib2@gmail.com, gerardajo944@gmail.com, and jboney2001@yahoo.fr. Mentor Ali Ber Lucien, Department of Microbiology, Quisqueya University, Port-au-Prince, Haiti, E-mail: lucienmentor@gmail.com. Eyal Leshem, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: wgp9@cdc.gov. S. Arunmozhi Balajee, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: fir3@cdc.gov. Mark A. Katz, Centers for Disease Control and Prevention Haiti, Port-au-Prince, Haiti, E-mail: markakatz@gmail.com. Michael D. Bowen, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: mkb6@cdc.gov.

REFERENCES

- Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI, 2009. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 200 (Suppl 1): S9–S15.
- Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD; WHO-coordinated Global Rotavirus Surveillance Network, 2012. 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* 12: 136–141.
- de Oliveira LH, Danovaro-Holliday MC, Sanwogou NJ, Ruiz-Matus C, Tambini G, Andrus JK, 2011. Progress in the introduction of the rotavirus vaccine in Latin America and the Caribbean: four years of accumulated experience. *Pediatr Infect Dis J* 30 (Suppl): S61–S66.
- WHO, 2008. *Estimated Rotavirus Deaths for Children Under 5 Years of Age: 2008*. Available at: http://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/en/.
- WHO, 2009. Rotavirus vaccines: an update. *Wkly Epidemiol Rec* 84: 533–537.
- Atherly DE, Lewis KD, Tate J, Parashar UD, Rheingans RD, 2012. Projected health and economic impact of rotavirus vaccination in GAVI-eligible countries: 2011–2030. *Vaccine* 30 (Suppl 1): A7–A14.
- Linhares AC, Velazquez FR, Perez-Schael I, Saez-Llorens X, Abate H, Espinoza F, López P, Macías-Parra M, Ortega-Barria E, Rivera-Medina DM, Rivera L, Pavía-Ruz N, Nuñez E, Damaso S, Ruiz-Palacios GM, De Vos B, O'Ryan M, Gillard P, Bouckennooghe A; Human Rotavirus Vaccine Study Group, 2008. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet* 371: 1181–1189.
- Rheingans RD, Constenla D, Antil L, Innis BL, Breuer T, 2007. Potential cost-effectiveness of vaccination for rotavirus gastroenteritis in eight Latin American and Caribbean countries. *Revista Panam Salud Publica* 21: 205–216.
- Steenland MW, Joseph GA, Lucien MA, Freeman N, Hast M, Nygren BL, Leshem E, Juin S, Parsons MB, Talkington DF, Mintz ED, Vertefeuille J, Balajee SA, Boney J, Katz MA, 2013. Laboratory-confirmed cholera and rotavirus among patients with acute diarrhea in four hospitals in Haiti, 2012–2013. *Am J Trop Med Hyg* 89: 641–646.
- Mijatovic-Rustempasic S, Tam KI, Kerin TK, Lewis JM, Gautam R, Quayle O, Gentsch JR, Bowen MD, 2013. Sensitive and specific quantitative detection of rotavirus A by one-step real-time reverse transcription-PCR assay without antecedent double-stranded-RNA denaturation. *J Clin Microbiol* 51: 3047–3054.
- Hull JJ, Teel EN, Kerin TK, Freeman MM, Esona MD, Gentsch JR, Cortese MM, Parashar UD, Glass RI, Bowen MD; National Rotavirus Strain Surveillance System, 2011. United States rotavirus strain surveillance from 2005 to 2008: genotype prevalence before and after vaccine introduction. *Pediatr Infect Dis J* 30 (Suppl): S42–S47.
- Levy K, Hubbard AE, Eisenberg JN, 2009. Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. *Int J Epidemiol* 38: 1487–1496.
- Bourdett-Stanziola L, Jimenez C, Ortega-Barria E, 2008. Diversity of human rotavirus G and P genotypes in Panama, Costa Rica, and the Dominican Republic. *Am J Trop Med Hyg* 79: 921–924.
- Bourdett-Stanziola L, Ortega-Barria E, Espinoza F, Bucardo F, Jimenez C, Ferrera A, 2011. Rotavirus genotypes in Costa Rica, Nicaragua, Honduras and the Dominican Republic. *Intervirology* 54: 49–52.

15. de Oliveira LH, Danovaro-Holliday MC, Andrus JK, de Fillipis AM, Gentsch J, Matus CR, Widdowson MA; Rotavirus Surveillance Network, 2009. Sentinel hospital surveillance for rotavirus in Latin American and Caribbean countries. *J Infect Dis* 200 (Suppl 1): S131–S139.
16. Ribas Mde L, Nagashima S, Calzado A, Acosta G, Tejero Y, Cordero Y, Piedra D, Kobayashi N, 2011. Emergence of G9 as a predominant genotype of human rotaviruses in Cuba. *J Med Virol* 83: 738–744.
17. Banyai K, Laszlo B, Duque J, Steele AD, Nelson EA, Gentsch JR, Parashar UD, 2012. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. *Vaccine* 30 (Suppl 1): A122–A130.