Journal of Medical Virology 81:2109–2113 (2009)

Rapid Changes in Rotaviral Genotypes in Ecuador

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Previous studies suggest that the emerging G9P[8] genotype was the most prevalent rotavirus genotype in Ecuador during 2005. This present study provides a temporal analysis of the distribution of rotavirus genotypes in two locations within Ecuador by adding additional years (2006 – early 2008) to the originally reported 2005 data. Data were collected in a rural (northern coastal Ecuador) and urban (Quito) area. In the rural area, a community sample of cases (those presenting diarrhea) and controls (those not presenting diarrhea) were collected between August 2003 and March 2008 resulting in a total of 3,300 stool samples (876 cases and 2,424 controls). Of these samples, 260 were positive for rotavirus by an immunochromatographic test (196 cases and 64 controls). In Quito, 59 fecal samples were collected from children presenting diarrhea and diagnosed with rotavirus. An RT-PCR analysis of samples collected between 2005 and 2007 suggested that G9 was replaced by G1 and G2 in the rural and urban settings. During this period G9 decreased from 79% to 9% while G2 increased from 0% to 43% in the rural communities, and G9 decreased from 79% to 37% while G2 increased from 3% to 57% in the urban area of Quito. This rapid replacement of G9 by G1 and G2 reinforces the necessity of surveillance to inform vaccination programs. ***J. Med. Virol. 81:2109–2113, 2009.***

KEY WORDS: rotavirus; genotype analysis; Ecuador; community study

INTRODUCTION

Rotavirus is an important cause of diarrhea responsible for 454,000–705,000 childhood deaths each year [Parashar et al., 2006]. This virus belongs to the *Reoviridae* family. It has a three-layered capsid that surrounds a genome made of 11 segments of double-stranded RNA. The middle capsid layer is composed of the VP6 protein, which is the most abundant protein and permits classification into five groups (A through E) based on the presence of specific epitopes. Most human-infecting rotaviruses belong to group A [Estes and Kapikian, 2007]. Proteins VP7 and VP4, also named G and P respectively, comprise the outermost layer of the capsid, and are used for typing purposes. The Rotavirus Classification Working Group has catalogued 20 VP7 genotypes and 28 VP4 genotypes [Matthijnssens et al., 2008; Solberg et al., 2009]. Globally, G1, G2, G3, G4, and G9 are the most prevalent VP7 genotypes, whereas P[8], P[4], and P[6] are the most prevalent VP4 genotypes [Gentsch et al., 2005; Santos and Hoshino, 2005]. The emerging G9 genotype has gained global importance during the past 10 years [Santos et al., 2001, 2005; Clark et al., 2004].

Rotavirus is diverse genotypically [Gentsch et al., 2005]. This diversity presents challenges in developing an effective vaccine. Available rotaviral vaccines have been shown to confer protection against gastroenteritis caused by genotypes homologous to those present in their formulations [Ruiz-Palacios et al., 2006; Vesikari et al., 2006], and a lower degree of protection against genotypes not present in vaccines [Ruiz-Palacios et al., 2006; Gurgel et al., 2007]. For example, these vaccine formulations do not include the G9 antigen; and although some vaccines have been shown to be protective for G9 genotypes, this may be largely due to the fact that G9 has been generally associated with P8, an antigen that is included in the vaccine formulations.

Two studies describing the rotavirus genotype distribution were conducted during 2005 in Ecuador [Endara et al., 2007; Naranjo et al., 2008]. Both studies showed that G9 was the most common genotype circulating throughout the country (10 provinces within the coastal, Andian, and Amazonian regions). The aim of

Grant sponsor: National Institute of Allergy and Infectious Disease (NIAID); Grant number: RO1-AI050038.

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Accepted 23 July 2009

DOI 10.1002/jmv.21632

Published online in Wiley InterScience (www.interscience.wiley.com)

this study is to present a temporal analysis of the distribution of the rotavirus genotypes circulating in 22 rural communities in the northern coast and in a children's hospital in Quito between 2005 and 2007.

METHODS

Between August 2003 and March 2008, 22 rural communities located in the northern coast of Ecuador were visited seven times in a rotational sequence. Each visit lasted for 15 days. Cases were defined as individuals with three or more loose stools within a 24 hr period and controls as individuals with no diarrhea within the past 6 days. Through a daily active surveillance of all households within a community, all cases of diarrhea were identified. For each case of diarrhea, three additional control stool specimens were randomly collected, one from within the case household and two from within the case community. Fecal samples obtained from the Hospital de Niños Baca Ortiz in Quito, from children presenting diarrhea and diagnosed with rotavirus, were collected between January 2007 and May 2007 to complement the 2005 samples previously collected [Endara et al., 2007]. A subset of the rural samples (from February 2005 to December 2007) and a random sample of 59 children (29 from 2005 and 30 from 2007) from the Quito hospital were genotyped using RT-PCR. All protocols were approved by the IRB committees of the University of Michigan and Universidad San Francisco de Quito.

Rotavirus Detection

All samples (symptomatic and non-symptomatic) were analyzed for the presence of rotavirus with a commercial immunochromatographic test (RIDA Quick Rotavirus, R-Biopharm AG).

Rotavirus Multiplex RT-PCR

The rotavirus-positive samples were preserved in liquid nitrogen and transported to Quito for PCR genotyping. The double-stranded rotavirus RNA was extracted from the stool specimens using TRIZOL Reagent (Invitrogen Corp., Carlsbad, CA) or the Ultra-Clean Tissue RNA Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to protocols supplied by the manufacturers. RNA was stored at −80°C until further use. A two-step, semi-nested multiplex RT-PCR was carried out for G- and P-genotyping. Briefly, primers *9con1* and *9con2* were used for the first amplification of the VP7 gene and primers *9T-1*, *9T-2*, *9T-3P*, *9T-4*, and *9T-9B* were then used to ascertain the G genotype [Das et al., 1994]. Primers *Con3* and *Con2* were used for the partial amplification of the VP4 gene and primers *1T-1*, *2T-1*, *3T-1*, *4T-1*, *5T-1*, and *ND2* were then used to ascertain the P genotype [Gentsch et al., 1992].

Viral RNA was denatured for 5 min at 97°C. Retrotranscription and the first amplification were carried out using a SuperScript III RT/Platinum Taq polymerase kit (Invitrogen Corp.). Primers were used at 200 nM

TABLE I. Age Distribution of Cases (Those With Diarrhea; N = 876) and Controls (Those Without Diarrhea; N = 2,424) From Rural Communities of Esmeraldas That Were Positive for Rotavirus (by the Immunochromatographic Test)

	Age group						
	Missing birth dates	<1	1–<5	5–<20	20–<40	>40	Total
Cases +/n (%)	24/96 (25.0)	45/133 (33.8)	71/398 (17.8)	30/131 (22.9)	5/39 (12.8)	21/79 (26.6)	196/876 (22.4)
Controls +/n (%)	2/145 (1.4)	1/44 (2.3)	9/254 (3.5)	26/974 (2.7)	18/508 (3.5)	8/499 (1.6)	64/2424 (2.6)
OR (95% CI)	23.8 (5.5, 103.7)	22.0 (2.9, 164.9)	5.9 (2.9, 12.1)	10.8 (6.2, 19.0)	4.0 (1.4, 11.4)	22.2 (9.4, 52.4)	10.6 (7.9, 14.3)

OR, odds ratio; CI, confidence interval.
Odd ratio is the odds of being a case relative to being a control. Samples were collected between August 2003 and March 2008. The number of positive (+) and tested cases (N) is reported. Confidence bands for the <1 year and 20–40 years age groups were obtained using the Fisher exact test.
+ Indicates number of rotavirus positive samples with the immunochromatographic test.

each, and the 1X buffer provided by the manufacturer contained 1.6 mM $MgSO_4$ and 200 μM of dNTPs. The retrotranscription was carried out at 42°C for 45 min and stopped at 96°C for 2 min. The first amplification consisted of 30 cycles at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 60 sec. The second amplification was carried out using PuReTaq Ready-To-Go™ PCR beads (Amersham Biosciences, Valley Stream, NY) and primers at a final concentration of 400 nM. The cycling parameters were 30 cycles at 94°C for 30 sec, 42°C for 30 sec and 72°C for 60 sec, and a final extension at 72°C for 1 min. Electrophoresis of the PCR product was run with 1.8% agarose gels at 60 V and visualized under UV light.

RESULTS

Between August 2003 and March 2008, a total of 3,300 stool samples were collected in 22 rural communities in the northern coast of Ecuador (876 cases and 2,424 controls). Of these, 260 were positive by the commercial immunochromatographic test (196 cases and 64 controls). The presence of diarrhea was significantly associated with rotavirus infection (odds ratio = 10.6; 95% CI: 7.9–14.3) (Table I), consistent with that reported for the 2005 samples (odds ratio = 9.2; 95% CI: 6.1–13.9) [Endara et al., 2007].

Of the 5 genotypes identified from the isolates, G9 represent 41% (90 of 220) while G1 and G2 represent 31% (68 of 220) of the samples (Table II). G1 and G2 had similar trends over time in contrast with the G9 genotype, which had an opposing trend. These trends were similar in both the rural communities of Esmeraldas as well as in the urban setting of Quito (Fig. 1). While G9 was dominant strain during the calendar year 2005 (present in 79% of the isolates in both Esmeraldas and Quito), it dropped within Esmeraldas to 28% and 9% during 2006 and 2007 respectively, and within Quito to 37% during 2007. The genotypes G1 and G2, on the other hand, was not identified in Esmeraldas during the calendar year 2005 but was present in 43% of the isolates in 2007. Similarly, in Quito, G2 was only isolated in 3% of the samples in 2005 but in 57% of the samples in 2007.

Ten percent of the samples had no detectable VP7 gene, which determines the G genotype, but did have a detectable VP4 gene providing an identification of the P genotype. Thirteen percent of the samples had no detectable VP4 or VP7 gene resulting in an untypeable classification. All but one of these untypeable isolates were from the Esmeraldas samples (Table II).

DISCUSSION

In a previous publication, data was presented showing that in 2005 G9 was the dominant strain circulating along the coast of Esmeraldas and in the Andean city of Quito [Endara et al., 2007]. This was consistent with another study describing rotavirus in urban settings in Ecuador [Naranjo et al., 2008]. There have been many other studies corroborating the emergence of G9 rotavirus in Latin America [Gentsch et al., 2005; Santos and Hoshino, 2005]. The genotypes G1 and G2, on the other hand, were not found in the 22 rural communities and were minor genotypes in samples from the urban hospital. The follow-up study presented here suggests that within 2 years, G9 was replaced by G1 and G2 in these same two regions of Ecuador. This increase in the G2 genotype is consistent with that observed in Brazil, El Salvador, Guatemala, Honduras, Argentina, and Paraguay [Leite et al., 2008].

Other studies have also shown that the prevalent rotavirus genotypes circulating within a population can change in short periods of time. In Thailand, during the years 2000–2001, the most prevalent genotype was G9; by the year 2003, G2 emerged as the prevailing genotype but only to decline again in 2004, when G1 became the most common genotype [Khamrin et al., 2007]. In Bangladesh between 2001 and 2005, the

TABLE II. Distribution of G and P Types of Rotavirus by Year in Rural Communities of Esmeraldas and in Quito Ecuador by Year[a]

	Number of strains detected					
	Esmeraldas			Quito		
Genotype	2005	2006	2007	2005	2007	Total
G1 (P[6], P_{NT})	3	17	1	1	0	22
G2 (P[4], P[8], P[9], P_{NT})	0	9	19	1	17	46
G3 P[6]	0	1	0	0	0	1
G4 P[6]	0	4	0	0	2	6
G9 (P[4], P[6], P[8], P_{NT})	34	19	4	23	10	90
G_{NT} (P[4], P[8], P[9])	2	9	11	0	0	22
G4/G9 P[6]	0	0	0	0	1	1
G2/G9 (P[8], P[4])	0	0	0	3	0	3
P_{NT} G_{NT}	7	9	12	1	0	29
Total	46	68	47	29	30	220

[a]As determined by genotype specific, multiplex reverse transcription-PCR. Samples were collected from 21 rural communities in Borbón Esmeraldas and from Quito, February 2005–December 2007. Undetermined types are designated NT. Coinfection with different genotypes is designated with a slash.

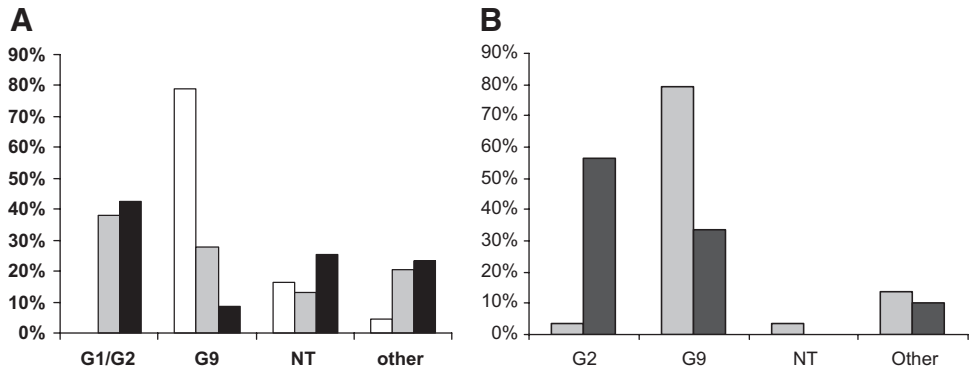

Fig. 1. Percent isolates identified as genotype G1 or G2, G9 by year (white: 2005, gray: 2006, black: 2007) from (**A**) Esmeraldas; (**B**) Quito. NT designates undetermined types for both G and P. "Other" includes genotypes G3, G4, or G_{NT} associated with a specific P type.

prevailing strains belonged to genotypes G1P[8] and G9P[8], however, in the 2005–2006 season G2P[4] became predominant [Rahman et al., 2005].

Changes in genotypic patterns within a population may be explained by the interplay between the population's immunity and viral evolution. The presence of antibodies against a given rotavirus serotype can prevent re-infections by the antigenically related viruses but not against other distinct serotypes [Franco et al., 2006]. Additionally, new highly infective viral strains may emerge only to disappear when populations reach herd immunity [Kobasa et al., 2004].

Some samples from the rural communities could not be genotyped. A previous study provided evidence that these isolates may be novel strains [Solberg et al., 2009]. This would suggest that the G9 strain was able to temporarily supplant these indigenous novel strains, explaining the higher proportion of isolates that could be typed during 2005 (Fig. 1A). The presence of unusual strains often occur in developing countries and are likely to occur due to human contact with animals [Gentsch et al., 2005; Franco et al., 2006]. These zoonotic infections can promote the emergence of new genotypes by viral reassortment.

In Ecuador two vaccines, the monovalent (Rotarix®) and pentavalent (Rotateq®) vaccines, are approved and are being used in vaccination campaigns. This sudden change in rotavirus genotypes prevalence over time reinforces the need for rotaviral surveillance studies while vaccination programs are ongoing.

ACKNOWLEDGMENTS

We thank the Ecologia, Desarrollo, Salud, y Sociedad (EcoDeSS) project field team for their invaluable contribution collecting the field data.

REFERENCES

Clark HF, Lawley DA, Schaffer A, Patacsil JM, Marcello AE, Glass RI, Jain V, Gentsch J. 2004. 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 the1995–1996 season. J Clin Microbiol 42:1434–1438.

Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI. 1994. Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol 32:1820–1822.

Endara P, Trueba G, Solberg OD, Bates SJ, Ponce K, Cevallos W, Matthijnssens J, Eisenberg JN. 2007. Symptomatic and subclinical infection with rotavirus P[8]G9, rural Ecuador. Emerg Infect Dis 13:574–580.

Estes MK, Kapikian AZ. 2007. Rotavirus. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, editors. Fields virology. Philadelphia: Lippincott Williams & Wilkins. pp 1917–1974.

Franco MA, Angel J, Greenberg HB. 2006. Immunity and correlates of protection for rotavirus vaccines. Vaccine 24:2718–2731.

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

Gentsch JR, Laird AR, Bielfelt B, Griffin DD, Banyai K, Ramachandran M, Jain V, Cunliffe NA, Nakagomi O, Kirkwood CD, Fischer TK, Parashar UD, Bresee JS, Jiang B, Glass RI. 2005. Serotype diversity and reassortment between human and animal rotavirus strains: Implications for rotavirus vaccine programs. J Infect Dis 192:S146–S159.

Gurgel RQ, Cuevas LE, Vieira SC, Barros VC, Fontes PB, Salustino EF, Nakagomi O, Nakagomi T, Dove W, Cunliffe N, Hart CA. 2007. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis 13:1571–1573.

Khamrin P, Peerakome S, Tonusin S, Malasao R, Okitsu S, Mizuguchi M, Ushijima H, Maneekarn N. 2007. Changing pattern of rotavirus G genotype distribution in Chiang Mai, Thailand from 2002 to 2004: Decline of G9 and reemergence of G1 and G2. J Med Virol 79:1775–1782.

Kobasa D, Takada A, Shinya K, Hatta M, Halfmann P, Theriault S, Suzuki H, Nishimura H, Mitamura K, Sugaya N, Usui T, Murata T, Maeda Y, Watanabe S, Suresh M, Suzuki T, Suzuki Y, Feldmann H, Kawaoka Y. 2004. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature 431:703–707.

Leite JP, Carvalho-Costa FA, Linhares AC. 2008. Group A rotavirus genotypes and the ongoing Brazilian experience: A review. Mem Inst Oswaldo Cruz 103:745–753.

Matthijnssens J, Ciarlet M, Rahman M, Attoui H, Banyai K, Estes MK, Gentsch JR, Iturriza-Gomara M, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Patton JT, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Desselberger U, Van Ranst M. 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol 153:1621–1629.

Naranjo A, Cedeno C, Teran E, Castello A. 2008. Prevalence of VP4 and VP7 genotypes of human rotavirus in Ecuadorian children with acute diarrhea. J Med Virol 80:1106–1111.

Parashar UD, Gibson CJ, Bresse JS, Glass RI. 2006. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 12:304–306.

Rahman M, Sultana R, Podder G, Faruque AS, Matthijnssens J, Zaman K, Breiman RF, Sack DA, Van Ranst M, Azim T. 2005. Typing of human rotaviruses: Nucleotide mismatches between the VP7 gene and primer are associated with genotyping failure. Virol J 2:24.

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, Salmeron J, Ruttimann R, Tinoco JC, Rubio P, Nunez E, Guerrero ML, Yarzabal JP, Damaso S, Tornieporth N, Saez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M. 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 354:11–22.

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

Santos N, Volotao EM, Soares CC, Albuquerque MC, da Silva FM, de Carvalho TR, Pereira CF, Chizhikov V, Hoshino Y. 2001. Rotavirus strains bearing genotype G9 or P[9] recovered from Brazilian children with diarrhea from 1997 to 1999. J Clin Microbiol 39:1157–1160.

Santos N, Volotao EM, Soares CC, Campos GS, Sardi SI, Hoshino Y. 2005. Predominance of rotavirus genotype G9 during the 1999, 2000, and 2002 seasons among hospitalized children in the city of Salvador, Bahia, Brazil: Implications for future vaccine strategies. J Clin Microbiol 43:4064–4069.

Solberg OD, Hasing ME, Trueba G, Eisenberg JN. 2009. Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus. Virology 385:58–67.

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. 2006. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 354:23–33.