Am. J. Trop. Med. Hyg., 85(5), 2011, pp. 957–960
doi:10.4269/ajtmh.2011.11-0401

Short Report: Rotavirus Prevalence in the Primary Care Setting in Nicaragua after Universal Infant Rotavirus Immunization

Sylvia Becker-Dreps,* Margarita Paniagua, Luis Enrique Zambrana, Filemon Bucardo, Michael G. Hudgens, David J. Weber, Douglas R. Morgan, and Félix Espinoza

Department of Family Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina; Department of Microbiology, Universidad Nacional Autónoma de Nicaragua, León, Nicaragua; Centro de Investigación en Demografía y Salud (CIDS), Universidad Nacional Autónoma de Nicaragua, León, Nicaragua; Department of Biostatistics, University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina; Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, North Carolina; Division of Gastroenterology, University of North Carolina School of Medicine, Chapel Hill, North Carolina

Abstract. Nicaragua was the first developing nation to implement universal infant rotavirus immunization with the pentavalent rotavirus vaccine (RV5). Initial studies of vaccine effectiveness in Nicaragua and other developing nations have focused on the prevention of hospitalizations and severe rotavirus diarrhea. However, rotavirus diarrhea is more commonly treated in the primary care setting, with only 1–3% of rotavirus cases receiving hospital care. We measured the prevalence of rotavirus infection in primary care clinics in León, Nicaragua, after introduction of the immunization program. In the post-vaccine period, 3.5% (95% confidence interval = 1.9–5.8) of children seeking care for diarrhea tested positive for rotavirus. A high diversity of rotavirus genotypes was encountered among the few positive samples. In conclusion, rotavirus was an uncommon cause of childhood diarrhea in this primary care setting after implementation of a rotavirus immunization program.

INTRODUCTION

Rotavirus causes an estimated 114 million cases of diarrhea in the world annually.[1] Approximately 1–3% of rotavirus cases receive care in the hospital, whereas 15–20% of cases receive care in the primary care setting.[1] Less severe cases of diarrhea, which are more likely to be treated in primary care clinics, are responsible for substantial morbidity.[2–4]

To reduce the burden of rotavirus diarrhea, a growing number of nations are adding the rotavirus vaccine to their national immunization schedules. In clinical trials conducted primarily in the United States and Europe, the pentavalent rotavirus vaccine (RV5) reduced clinic visits for rotavirus diarrhea by 86%.[5] Subsequent clinical trials and effectiveness studies in developing nations show less robust protection; these studies have focused on the prevention of severe disease and hospital visits.[6–9] Less is known about the vaccine's impact on the primary care setting in the developing world.

In October of 2006, Nicaragua became the first developing nation to initiate universal infant rotavirus immunization with RV5. Infants are offered the vaccine at 2, 4, and 6 months of age as part of Nicaragua's Expanded Program on Immunization (EPI). Before the rotavirus immunization program in Nicaragua, rotavirus was isolated among 28.4% of children seeking care for diarrhea in a combination of primary and secondary healthcare facilities.[10] After the immunization program, a study based in Nicaraguan hospitals found a vaccine effectiveness of 46% against hospitalization for rotavirus diarrhea.[6] Also, Ministry of Health (MOH) data from health facilities show a 15% decrease in diarrhea visits among infants during the rotavirus season.[11]

The aim of this study was to measure the prevalence of rotavirus infection in the primary care setting in León, Nicaragua, after RV5 introduction. Secondary aims included examining risk factors for rotavirus infection and also characterizing rotavirus genotypes circulating in the post-vaccine era.

*Address correspondence to Sylvia Becker-Dreps, Department of Family Medicine, University of North Carolina School of Medicine, 590 Manning Dr., Chapel Hill, NC 27599-7599. E-mail: sbd@unc.edu

MATERIALS AND METHODS

Study design and setting. We conducted surveillance for rotavirus infection among children receiving care for diarrhea at six government-administered primary care clinics distributed throughout León, Nicaragua. The study was conducted over a 12-month period from April of 2008 to March of 2009.

León is Nicaragua's second largest city, with an estimated population of 192,000 people in 2009. During the time of the study, the MOH reported that RV5 coverage in León was 98%, 93%, and 77% for receipt of the first, second, and third doses, respectively. The government-administered primary care clinics in León are free of charge and provide preventive and acute care. The clinics have capacity for intravenous hydration, but children requiring inpatient management are transferred to León's University Hospital. The MOH estimates that 90% of the population receives care in these clinics, whereas 10% receives care from private physicians.

A rotavirus prevalence study was conducted in seven government-administered primary care clinics in León during a 12-month period (August of 2002 to July of 2003) before vaccine introduction. Using the same recruitment criteria as the present study, the pre-vaccine study found a rotavirus prevalence among children with diarrhea of 14.0% (69/493; 95% confidence interval [CI] = 10.9–17.1).[12] For all health facilities in León during this pre-vaccine year studied, the MOH reported 2,745 diarrhea visits among children under the age of 5 years. During the present year studied, the MOH reported 2,494 diarrhea visits among children under the age of 5 years.

Recruitment and questionnaire administration. Children ages 10 weeks to 36 months with an onset of diarrhea within 14 days before the clinic visit were recruited. Diarrhea was defined as more than or equal to three loose or liquid stools over a 24-hour period. Two study nurses were present at each site and recruited eligible children during clinic work hours. Each clinic is required to report numbers of diarrhea visits to the MOH. We used these reports to estimate our recruitment rate.

Study nurses administered a questionnaire to the parent(s) that assessed participant characteristics and obtained anthropometric measurements and temperature. A detailed vaccine

history was obtained from the medical record. The study received Institutional Review Board approvals from the University of Nicaragua, León, Nicaragua (UNAN-León), and the University of North Carolina, Chapel Hill, NC. Informed consent was obtained from a parent or legal guardian.

Stool analysis. Stool samples were collected in sterile containers or from soiled diapers during the clinic visit or within 3 days after the visit, and they were transported to the UNAN-León microbiology laboratory at 4°C. A commercial enzyme-linked immunosorbent assay (ELISA; Oxoid, Cambridge, United Kingdom) was used for identification of rotavirus. G (G1, G2, G3, G4, G8, G9, G10, and G12) and P (P4, P6, P8, P9, P10, and P11) genotypes were investigated by reverse transcription–polymerase chain reaction (RT-PCR) using primers and methods previously described.[13]

Statistical analysis. The prevalence of rotavirus was estimated by the proportion of samples that tested positive for rotavirus. The corresponding exact 95% CI was calculated assuming that samples from the same child were independent. Characteristics of children and diarrhea episodes with and without rotavirus were compared using the Wilcoxon rank sum test or Fisher's exact test. In the analysis of child characteristics, a child who provided two samples was classified by the result of the first sample. Each child's weight for age percentile was calculated using World Health Organization standards. Children were considered up to date if they received all recommended vaccine dosages for their age without being more than 1 month behind. STATA 9 software (College Station, TX) was used for all analyses.

RESULTS

Overall, 392 children presenting with diarrhea were enrolled in the study. Eighteen children presented with two diarrhea episodes for a total of 410 episodes. These 410 episodes among children under age 3 years represented 59% of the diarrhea visits reported to the MOH in the under age 5 years age group from these six clinics; therefore, our recruitment rate was at least 59%. Stool samples were obtained for 403 of 410 episodes (98.3%). Five children who were not able to provide a stool sample were excluded from the analysis.

Fourteen of four hundred three (3.5%; 95% CI = 1.9–5.8) stool samples tested positive for rotavirus, of which four samples were P[8]G1, two samples were P[4]G2, one sample was P[8]G3, one sample was P[6]G4, and three samples were mixed infections. One sample was negative for the genotypes investigated, and two samples were not investigated for genotypes because of insufficient stool quantity. The three mixed infections were P[4]:G2/G9, P[8]/P[4]:G1/G9, and P[8]:G1/G2/G4. Among those children who tested positive for rotavirus, 10 children had received all three doses of the vaccine, 3 children were partially immunized, and 1 child had not received vaccine. All of the G and P types reported above were observed among fully immunized children. The child with the P[8]:G1/G2/G4 infection was unimmunized. Two children presented with rotavirus diarrhea within 2 days after receiving the vaccine.

Characteristics of the children are shown in Table 1. Children had an average age of 12.4 months, and 71.9% were breastfed. All participants who had received RV5 had also received the oral polio vaccine on the same day for at least one RV5 dose. The children who tested positive for rotavirus were less likely to have an indoor toilet. There was no difference in immunization status between those children who tested positive for rotavirus and those children with diarrhea of another cause.

Clinical characteristics of the diarrhea episodes experienced among the children are shown in Table 2. There were no differences in these clinical characteristics among episodes caused by rotavirus and episodes caused by other etiologies. There were also no differences in clinical characteristics of rotavirus episodes between those children immunized and those children unimmunized with RV5.

DISCUSSION

Rotavirus infection was uncommon among children seeking care for diarrhea in the primary care setting in León, Nicaragua. The low prevalence of rotavirus infection (3.5%; 95% CI = 1.9–5.8) occurred despite high rates of breastfeeding and co-administration of the oral polio vaccine, factors that are hypothesized to contribute to the lower effectiveness of the rotavirus vaccine in developing world settings.[14] The rotavirus prevalence that we encountered was lower than reported in pre-vaccine studies conducted in the primary care setting in the same city[12] and other Latin American countries (prevalence range = 14–35%).[15–18] We also confirmed broad coverage

TABLE 1
Characteristics of 387 children with diarrhea in the primary care setting

Characteristic	All children (N = 387)	Children with rotavirus (N = 12)*	Children with diarrhea of other cause (N = 372)*	P value comparing children with and without rotavirus
Mean age (months)	12.4 (SD = 8.2)	14.3 (SD = 9.3)	12.3 (SD = 8.2)	0.50
Sex (male)	50.9% (197/387)	50.0% (6/12)	50.8% (189/372)	1.00
Maternal education above elementary school level	64.2% (247/385)	41.7% (5/12)	64.9% (240/370)	0.13
Daycare attendance	3.9% (15/385)	0.0% (0/12)	4.1% (15/370)	1.00
In home municipal water supply	91.2% (353/387)	83.3% (10/12)	91.4% (340/372)	0.29
Indoor toilet	46.8% (181/387)	16.7% (2/12)	47.3% (176/372)	0.042
Currently breastfed	71.9% (261/363)	60.0% (6/10)	72.9% (255/350)	0.47
Mean weight for age (percentile)†	45.4 (SD = 30.3)	48.3 (SD = 28.1)	45.6 (SD = 30.4)	0.73
Rotavirus vaccine (three doses)	57.1% (221/387)	66.7% (8/12)	56.5% (210/372)	0.57
Rotavirus vaccine (up to date)‡	90.7% (313/345)	100.0% (11/11)	90.3% (299/331)	0.61
Same day oral polio vaccine§	100.0% (334/334)	100.0% (11/11)	100.0% (320/320)	1.00

SD = standard deviation.
*For the 18 children presenting with two diarrhea episodes, categorization is based on the first stool sample result. The total number in the two groups does not sum to 387 children, because 3 children had indeterminate stool results.
†World Health Organization weight for age growth standards.
‡Among those children who were eligible to receive the vaccine.
§Among those children who received any of the three doses of the rotavirus vaccine.

TABLE 2
Characteristics of diarrhea episodes ($N = 403$)

Characteristic	All episodes ($N = 403$)	Rotavirus episodes ($N = 14$)*	Diarrhea of other cause ($N = 386$)*	*P* value comparing episodes with and without rotavirus
Diarrhea duration before clinic visit (mean days)	2.8 (SD = 1.9)	2.8 (SD = 1.7)	2.8 (SD = 1.9)	0.84
Maximum number of stools within 24 hours (mean)	5.4 (SD = 2.3)	4.6 (SD = 1.2)	5.4 (SD = 2.3)	0.21
Vomiting	39.2% (158/403)	57.1% (8/14)	38.6% (149/386)	0.18
Fever (≥ 38.3°C)	4.4% (17/384)	0.0% (0/12)	4.6% (17/370)	1.0
Received ORS before clinic visit	33.7% (136/403)	50.0% (7/14)	33.4% (129/386)	0.25
Received intravenous hydration	2.7% (11/401)	0.0% (0/14)	2.9% (11/384)	1.0

ORS = oral rehydration solution; SD = standard deviation.
*Total does not sum to 403 results because of three indeterminate stool results.

of the rotavirus vaccine among the children in the study. By distributing the vaccine through Nicaragua's EPI, 90.7% of the children were up to date on the vaccine.

We observed high genetic diversity among the few rotavirus-positive samples, which was also observed before vaccine introduction in Central America.[19–21] In addition, we observed that, among the fully immunized children who developed rotavirus infection, one-half were infected with P types not included in RV5. Finally, we observed a high proportion of mixed infections or infections with more than one rotavirus genotype. Mixed infections may allow for exchange of genetic material, resulting in unusual strains of unknown pathogenic potential.[22] Future surveillance is warranted to investigate whether any genotypes are increasing in incidence in the population.

Although the vaccine's effectiveness in the primary care setting is supported by the low rotavirus prevalence compared with pre-vaccine studies, we did not find a statistical difference in immunization status between children with and without rotavirus diarrhea. Our study may have been limited in power to detect such a difference. An additional study limitation includes the inherent annual variation in rotavirus prevalence. Lastly, 10.9% of the participants were ineligible to receive the vaccine because of age greater than 2 months when the immunization program was introduced. In this group, any changes in rotavirus prevalence may be because of indirect effects of the vaccine.

In conclusion, rotavirus was not a common cause of childhood diarrhea in this Central American primary care setting after the introduction of a rotavirus immunization program. Future studies are warranted to investigate diarrhea etiologies in the post-rotavirus vaccine era, to both help direct clinicians in the appropriate treatment of children with diarrhea and guide the choice of future vaccine candidates and prevention efforts.

Received June 22, 2011. Accepted for publication August 19, 2011.

Acknowledgments: We would like to thank Mercedes Delgadillo, Dr. Xiomara Toruño, and the staff of the Centro de Investigación en Demografía y Salud (CIDS) for their contributions to this study. We would also like to thank Dr. Gilberto Moreno, Sistemas Locales de Atención Integral a la Salud (SILAIS), and the primary care nurses who assisted with the study.

Financial support: This research was supported by a grant from the Gorgas Memorial Institute. Sylvia Becker-Dreps is supported by Grant 4K01TW008401-03 from the National Institutes of Health Fogarty International Center.

Disclaimer: Some of the authors were awarded investigator-initiated research grants from Merck or served as consultants for Merck for unrelated studies. This statement is made in the interest of full disclosure and not because the authors consider this relationship to be a conflict of interest.

Authors' addresses: Sylvia Becker-Dreps, Department of Family Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, E-mail: sbd@unc.edu. Margarita Paniagua and Filemon Bucardo, Departamento de Microbiología, Campus Médico Universidad Nacional Autónoma de Nicaragua, León, Nicaragua, E-mails: paniaguagaitan@yahoo.com.mx and fili_bucardo@hotmail.com. Luis Enrique Zambrana, Centro de Investigación en Demografía y Salud (CIDS), Universidad Nacional Autónoma de Nicaragua, León, Nicaragua, E-mail: lamarcoleta@gmail.com. Michael G. Hudgens, Department of Biostatistics, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, E-mail: mhudgens@email.unc.edu. David J. Weber, Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, E-mail: Dweber@unch.unc.edu. Douglas R. Morgan, Division of Gastroenterology, University of North Carolina School of Medicine, Chapel Hill, NC, E-mail: douglas_morgan@med.unc.edu. Félix Espinoza, Centro de Enfermedades Infecciosas (CEI), Departamento de Microbiología, Campus Médico Universidad Nacional Autónoma de Nicaragua, León, Nicaragua, E-mail: espinozafelix08@gmail.com.

REFERENCES

1. Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, Jiang B, Gentsch JR, 2006. Rotavirus vaccines: current prospects and future challenges. *Lancet 368:* 323–332.
2. Guerrant RL, Schorling JB, McAuliffe JF, de Souza MA, 1992. Diarrhea as a cause and an effect of malnutrition: diarrhea prevents catch-up growth and malnutrition increases diarrhea frequency and duration. *Am J Trop Med Hyg 47:* 28–35.
3. Niehaus MD, Moore SR, Patrick PD, Derr LL, Lorntz B, Lima AA, Guerrant RL, 2002. Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. *Am J Trop Med Hyg 66:* 590–593.
4. Lorntz B, Soares AM, Moore SR, Pinkerton R, Gansneder B, Bovbjerg VE, Guyatt H, Lima AM, Guerrant RL, 2006. Early childhood diarrhea predicts impaired school performance. *Pediatr Infect Dis J 25:* 513–520.
5. 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; Rotavirus Efficacy and Safety Trial (REST) Study Team, 2006. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med 354:* 23–33.
6. Patel M, Pedreira C, De Oliveira LH, Tate J, Orozco M, Mercado J, Gonzalez A, Malespin O, Amador JJ, Umaña J, Balmaseda A, Perez MC, Gentsch J, Kerin T, Hull J, Mijatovic S, Andrus J, Parashar U, 2009. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA 301:* 2243–2251.
7. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, Ngwira B, Victor JC, Gillard PH, Cheuvart BB, Han HH, Neuzil

KM, 2010. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med 362:* 289–298.
8. Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ, Podder G, Vu DT, Le TP, Luby SP, Le HT, Coia ML, Lewis K, Rivers SB, Sack DA, Schödel F, Steele AD, Neuzil KM, Ciarlet M, 2010. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet 376:* 615–623.
9. Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, Binka FN, Steele AD, Laserson KF, Ansah NA, Levine MM, Lewis K, Coia ML, Attah-Poku M, Ojwando J, Rivers SB, Victor JC, Nyambane G, Hodgson A, Schödel F, Ciarlet M, Neuzil KM, 2010. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet 376:* 606–614.
10. Espinoza F, Paniagua M, Hallander H, Hedlund KO, Svensson L, 1997. Prevalence and characteristics of severe rotavirus infections in Nicaraguan children. *Ann Trop Paediatr 17:* 25–32.
11. Becker-Dreps S, Paniagua M, Dominik R, Cao H, Shah NK, Morgan DR, Moreno G, Espinoza F, 2011. Changes in childhood diarrhea incidence in Nicaragua following 3 years of universal rotavirus immunization. *Pediatr Infect Dis J 30:* 243–247.
12. Espinoza F, 2004. Rotavirus in pediatric gastroenteritis in Nicaraguan children. PhD thesis. Karolinska Institutet, Stockholm, Sweden.
13. Iturriza-Gómara M, Kang G, Gray J, 2004. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *J Clin Virol 31:* 259–265.
14. Parashar UD, Glass RI, 2009. Rotavirus vaccines—early success, remaining questions. *N Engl J Med 360:* 1063–1065.
15. O'Ryan M, Díaz J, Mamani N, Navarrete M, Vallebuono C, 2007. Impact of rotavirus infections on outpatient clinic visits in Chile. *Pediatr Infect Dis J 26:* 41–45.
16. Carmona RC, Timenetsky Mdo C, da Silva FF, Granato CF, 2004. Characterization of rotavirus strains from hospitalized and outpatient children with acute diarrhoea in São Paulo, Brazil. *J Med Virol 74:* 166–172.
17. Cama RI, Parashar UD, Taylor DN, Hickey T, Figueroa D, Ortega YR, Romero S, Perez J, Sterling CR, Gentsch JR, Gilman RH, Glass RI, 1999. Enteropathogens and other factors associated with severe disease in children with acute watery diarrhea in Lima, Peru. *J Infect Dis 179:* 1139–1144.
18. Pérez-Schael I, González R, Fernández R, Alfonzo E, Inaty D, Boher Y, Sarmiento L, 1999. Epidemiological features of rotavirus infection in Caracas, Venezuela: implications for rotavirus immunization programs. *J Med Virol 59:* 520–526.
19. Espinoza F, Bucardo F, Paniagua M, Svensson L, Hallander HO, Bondeson K, 2006. Shifts of rotavirus G and P types in Nicaragua—2001–2003. *Pediatr Infect Dis J 25:* 1078–1080.
20. 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.
21. Bourdett-Stanziola L, Jiménez 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.
22. 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 (Suppl 1):* S146–S159.