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Surveillance of Rotavirus Strains in the United States: Identification of Unusual Strains

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Rotavirus strains from 964 fecal specimens collected from children at 11 U.S. hospital laboratories from November 1997 to March 1998 and from samples collected at 12 laboratories from November 1998 to March 1999 were typed for G and P proteins. Serotype G1 was the predominant serotype in 1997–1998 (88%), followed by G2 (6.2%), G9 (3.3%), and G3 (1.5%). This pattern was similar to that seen in 1998–1999: G1 (79%), G2 (15%), G9 (3.0%), G4 (1.6%), and G3 (0.3%). Novel P[9] strains were identified in both seasons, and analysis of a 364-nucleotide fragment from gene segment 4 of one of the strains demonstrated 97.3% nucleotide identity with the prototype P3[9],G3 strain, AU1, isolated in Japan. This is the first report of a human AU1-like strain in the United States. These results reinforce our initial findings that serotype G9 persists in the United States but has not become a predominant strain and that the common serotypes G1 to G4 account for almost 90% of strains in circulation. Other uncommon strains exist in the United States but may have been overlooked before because of their low prevalence and the use of inadequate diagnostic tools.

Human rotavirus is the most common etiologic agent of severe diarrhea in young children worldwide (14). Vaccines under development hold the promise of substantially reducing the severe disease caused by rotavirus infection. The first vaccines have been developed to provide specific protection against the four predominant serotypes of rotavirus, G1 to G4 (15). Since less-common strains are in circulation, knowledge of rotavirus strains in current circulation will aid in assessing whether candidate vaccines will protect against these serotypes as well.

The rotavirus genome is composed of 11 segments of double-stranded RNA located inside the core of a triple-layered protein capsid. Each gene segment encodes a specific viral protein, of which six are structural (VP1 to VP6) and five are nonstructural (NSP1 to NSP5) (5). The two viral outer capsid proteins, VP4 and VP7, elicit a neutralizing immune response, creating both serotype-specific and cross-reactive immunity (12, 21). These proteins are also the basis of the G (VP7 glycoprotein) and P (protease-activated VP4 protein) serotypes. To date, 9 P serotypes and 10 G serotypes have been identified in humans by cross-neutralization tests (5, 21a, 26, 28). Genotyping and serotyping studies indicate that only four frequently observed neutralization antigen gene combinations—P[8],G1; P[4],G2; P[8],G3; and P[8],G4—are common worldwide (7), although large regional variation of G serotypes (e.g., G5 in Brazil and G9 in India) has been documented in some developing countries (8, 23).

In 1996, the Centers for Disease Control and Prevention established the National Rotavirus Strain Surveillance System to document the serotypes in circulation before and after the implementation of a U.S. rotavirus vaccination program (24) and to determine whether uncommon strains not represented in the vaccine might emerge to become more prevalent following widespread use of the vaccine in children. During the first year of surveillance and before vaccinations began, our lab group unexpectedly found a relatively high prevalence of serotype G9 in 4 of 10 U.S. cities (24). Together with other recent reports of elevated rates of serotype G9 in children from India, Bangladesh, France, Malawi, Australia, and the United Kingdom, these results raised the possibility that serotype G9 might represent an emerging strain that could escape vaccine-induced immunity and become more prevalent after the start of the vaccination program in the fall of 1998 (1, 3, 3a, 22, 23, 30; M. Iturriza, J. Green, M. Ramsay, D. Brown, U. Desselberger, and J. J. Gray, Abstr. 18th Am. Soc. Virol., abstr. W43-2, 1999).

We report here the results of 2 additional years of rotavirus strain surveillance in the United States, including the first year of the vaccination program (1998–1999), and compare these with the results from the first year of surveillance.

During the U.S. rotavirus season, November to March, a total of 325 rotavirus-positive diarrhea specimens were collected from children at 11 hospital-based laboratories in the United States in 1997–1998, and 639 specimens were received from 12 collaborators in 1998–1999, using a protocol described

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TABLE 1. G types identified in U.S. rotavirus strain surveillance years 1997–1998 and 1998–1999[a]

Origin of isolate	No. of strains tested	% of strains classified as:						
		G1	G2	G3	G4	G9	Mixed infections[b]	NT[c]
Atlanta, Ga.	17/58	82/67	12/5.2	—[d]/—	—/10.3	5.9/17	—/—	—/—
Corpus Christi, Tex.	32/NC[e]	97/NC	—/NC	3.1/NC	—/NC	—/NC	—/NC	—/NC
Denver, Colo.	26/14	81/64	12/36	—/—	—/—	7.7/—	—/—	—/—
East Meadow, N.Y.	NC/28	NC/79	NC/3.6	NC/—	NC/—	NC/18	NC/—	NC/—
Indianapolis, Ind.	24/38	83/97	17/—	—/—	—/—	—/—	—/—	—/2.6
Kansas City, Mo.	70/96	100/78	—/19	—/—	—/—	—/—	—/1.0	—/1.0
Little Rock, Ark.	2/5	50/—	50/80	—/—	—/—	—/—	—/20	—/—
Newark, Del.	3/16	100/56	—/6.3	—/—	—/25	—/13	—/—	—/—
Omaha, Nebr.	46/44	78/89	4.3/6.8	4.3/2.3	—/—	10.8/2.3	2.2/—	—/—
Philadelphia, Pa.[f]	32/12	91/33	—/58	3.1/—	—/—	3.1/—	—/8.3	3.1/—
Reno, Nev.	NC/114	NC/90	NC/9.6	NC/—	NC/—	NC/—	NC/—	NC/—
San Diego, Calif.	42/159	86/74	9.5/25	—/—	—/—	4.8/0.6	—/0.6	—/—
Seattle, Wash.	31/55	84/89	13/7.3	3.2/—	—/—	—/—	—/—	—/3.2
Total	325/639	88.6/78.9	6.2/15	1.5/0.3	0/1.6	3.3/3.0	0.3/0.6	0.3/0.6

[a] Data shown are data for the 1997–1998 surveillance period/data for the 1998–1999 surveillance period.
[b] Strains classified as mixed infections included one P[8],G(1+9) strain from 1997–1998 and two P[4],G(1+2) strains and two P[4+8],G(1+2) strains from 1998–1999.
[c] NT, nontypeable.
[d] —, no samples were identified as this type.
[e] NC, specimens were not collected from this site.
[f] In 1998–1999, only the first few specimens were received and analyzed.

previously (24). In brief, each hospital sent rotavirus-positive specimens that were confirmed to be positive with a commercial rotavirus detection kit (Rotaclone; Meridian Diagnostics, Inc., Cincinnati, Ohio) at the Centers for Disease Control and Prevention. All rotavirus-positive samples were tested for G-serotype and VP6-subgroup antigens by using a monoclonal antibody (MAb)-based enzyme immunoassay (EIA) (2, 11, 27). Samples that could not be G serotyped by EIA were genotyped by reverse transcription-PCR (RT-PCR) (4, 9). Using multiplex seminested RT-PCR (6), we P genotyped a subset of G1 strains (41% in 1998 and 32% in 1999) because they were very abundant. In contrast, all P genotypes were determined for each of the less prevalent types G2, G3, G4, and G9. Subsets of serotype G1 samples for P genotyping were selected systematically in the database, ensuring that at least 25% of G1 strains representative of collection dates from each locale were analyzed. These results were then extrapolated to provide a P genotype for 100% of the G1 strains. Classifications of P and G types were designated in accordance with recommendations of the Rotavirus Nomenclature Working Group (5). To confirm the P genotype of the AU1-like strain, P[9],G3, a fragment of the VP4 gene was sequenced by using the primer pair (con2 and con3) which amplifies an 876-nucleotide segment in the VP8 region with methods described previously (24).

Of the 964 rotavirus specimens examined for G and P types, five samples represented G mixed infections and only five could not be G typed (Table 1). Serotype G1 predominated each year in all cities, except for Little Rock and Philadelphia in 1998–1999, at a prevalence of 50 to 100% per locale, constituting 89% of specimens in 1997–1998 and 79% of specimens in 1998–1999. Other G types varied by location, with G2 being the second most common overall during both years (6.2 and 15% in 1997–1998 and 1998–1999, respectively); however, in Little Rock, Ark., and Philadelphia, Pa., type G2 was more prevalent than G1 in 1998–1999. Interestingly, strains of the novel G9 serotype were the third most prevalent in the second (3.3%) and third (3.0%) years of surveillance. Other G serotypes (G3 and G4) were less abundant and occurred sporadically year to year.

During 3 years of surveillance, 1,316 rotavirus strains could be classified as 4 common and 6 uncommon serotypes, excluding nontypeables and mixed infections (Table 2). Consequently, the four strains that are common globally (P[8],G1; P[8],G3; P[8],G4; and P[4],G2) represented almost 90% of the total

TABLE 2. Rotavirus strains identified during three rotavirus seasons from 1996 to 1999

Rotavirus strain	% of strains (*n*)			
	1996–1997[a]	1997–1998[b]	1998–1999[b]	Total[b]
Common strains				
P[8],G1	67.9 (239)	82.3 (267)	76.4 (488)	75.5 (994)
P[8],G3	8.0 (28)	0.6 (2)	0.16 (1)	2.4 (31)
P[8],G4	1.1 (4)	0	1.6 (10)	1.1 (14)
P[4],G2	8.2 (29)	6.2 (20)	14.7 (94)	10.9 (143)
Subtotal				89.8 (1,182)
Uncommon strains				
P[4],G1	0.3 (1)	0	0	0.1 (1)
P[6],G1	1.1 (4)	0.8 (3)	0	0.5 (7)
P[6],G9	5.4 (19)	1.8 (6)	2.7 (17)	3.2 (42)
P[8],G2	0.3 (1)	0	0	0.1 (1)
P[8],G9	2.3 (8)	1.5 (5)	0.3 (2)	1.1 (15)
P[9],G3	0	0.3 (1)	0.16 (1)	0.2 (2)
Other	5.4 (19)	6.5 (21)	4.1 (26)	5.0 (66)
Subtotal				10.2 (134)
Total	100.0 (352)	100.0 (325)	100.1 (639)	100.1 (1316)

[a] Data for 1996–1997 were taken from Ramachandran et al. (24).
[b] Data were based on extrapolations of a subset of G1s that were P genotyped: 41% in 1997–1998 and 32% in 1998–1999.
[c] G and/or P mixed infections were detected in each year: 1.4% in 1996–1997, 0.3% in 1997–1998, and 3.4% in 1998–1999. G and/or P nontypeables were also identified yearly: 4.0% in 1996–1997, 6.2% in 1997–1998, and 0.6% in 1998–1999.

TABLE 3. Rotavirus strains identified as P[6],G9 and P[8],G9 from 1996 to 1999 in the United States

City	% of strains (*n*)					
	1996–1997		1997–1998		1998–1999	
	P[6],G9	P[8],G9	P[6],G9	P[8],G9	P[6],G9	P[8],G9
Atlanta, Ga.	NC[a]	NC	5.9 (1)		17.2 (10)	
Denver, Colo.			7.7 (2)			
East Meadow, N.Y.	NC	NC	NC	NC	17.9 (5)	
Indianapolis, Ind.	26.1 (6)	21.7 (5)				
Kansas City, Mo.	12.9 (10)	1.3 (1)				
Little Rock, Ark.	18.2 (2)					
Newark, Del.					12.5 (2)	
Omaha, Nebr.	1.9 (1)	3.8 (2)		10.9 (5)		2.3 (1)
Philadelphia, Pa.			3.1 (1)			
San Diego, Calif.			4.8 (2)			0.6 (1)
Total	5.4 (19)	2.3 (8)	1.8 (6)	1.5 (5)	2.7 (17)	0.3 (2)

[a] Data were not collected (NC) from Atlanta or East Meadow during this period of surveillance.

strains, with some annual variability. The remaining 10.3% of strains were characterized as P[4],G1; P[6],G1; P[6],G9; P[8],G2; P[8],G9; P[9],G3; G and P mixed infections; and nontypeables. A number of G9 strains were found each year in addition to single strains of those mentioned above. The P[9] strains (long E-type and subgroup-I antigens) mentioned here were originally isolated in Japan (20) and to our knowledge are the first identified in the United States. On the basis of our preliminary findings in 1998–1999, the period after vaccination began, no marked difference was identified in the pattern of rotavirus strain prevalence for that year.

The most intriguing result for the 1996–1997 rotavirus season was the presence of serotype G9 in the United States at a frequency of 7.7%, making it the fourth most-prevalent G type for that year (Table 3). Prior to 1996, only one G9 strain had been isolated in the United States despite much screening, and that was reported 13 years ago (1a). Since the first year of surveillance, G9 strains have not disappeared but have persisted as two variants, P[8],G9 and P[6],G9, at a decreased prevalence of 3.3% in 1997–1998 and 3.0% in 1998–1999. G9 strains have been identified at 10 of the 13 locales surveyed and were detected in Omaha during each consecutive year of surveillance. Our results are consistent with works in progress in several laboratories where G9 strains have been isolated in the United States in recent years (F. E. Campos, P. Azimi, M. A. Staat, T. Berke, L. J. Jackson, D. I. Bernstein, D. Ward, L. K. Pickering, and D. O. Matson, Abstr. 37th Infect. Dis. Soc. Am. (IDSA), abstr. 702, 1999; V. Jain, H. F. Clark, P. Dennehy, K. Zangwill, C. D. Kirkwood, R. I. Glass, and J. R. Gentsch, Abstr. 18th Amer. Soc. Virol., abstr. W43-4, 1999). The serotype P[6],G9 strains identified during the 3 years of surveillance had a short electropherotype by polyacrylamide gel electrophoresis and VP6 subgroup-I antigens and were distinct from serotype P[8],G9, which had a long electropherotype and VP6 subgroup-II antigens. In contrast to the findings of Ramachandran et al. (24), the serotype G9 strains found in 1997–1999 did not react with MAb F45:8 in EIA, suggesting either some antigenic drift in the G9 strains or the weak reactivity of the F45:8 MAb (16). RT-PCR, using G9 specific primers, provided the sole means to identify G9 strains (4).

In conclusion, as part of the National Rotavirus Strain Surveillance System, over 1,300 rotavirus strains from 3 consecutive years of rotavirus surveillance have been typed, and only 10 specimens were found to be G nontypeable. The common G1 to G4 strains represented the majority of the strains each year; however, some unusual strains were identified as well. It is possible that rare strains were detected because of increased sample size and improved diagnostics for rotavirus characterization. Prior to this study and three reports in the last year (24; Jain et al., Abstr. 18th Amer. Soc. Virol.; Campos et al., Abstr. 37th IDSA), only four G serotypes were identified in the United States, with the exception of a single G9 isolate found in Philadelphia in 1983 (1a, 10, 19, 25); we have now identified a fifth serotype, G9, that appears to be endemic in U.S. children at an average prevalence of 4.3%. Overall, we can detect 10 P and G type combinations, although 6 of these are infrequently identified but nevertheless present. These results extend previous findings demonstrating the presence of rotavirus with uncommon P and G combinations in the United States (18, 25). Rotavirus mixed infections also existed but at a lower rate than levels found in India and Brazil. Since mixed infections provide the means for natural reassortment and virus evolution, it is likely that in the United States, virus evolution is slower than in countries like India and Brazil, where mixed infections are more common (17, 23, 29; V. Gouvea and N. Santos, Letter, Vaccine **17:**1291–1292, 1999).

This study has several limitations, including the small number of sites surveyed, the finite number of strains typed, and the number of genes (VP7 and VP4) used to characterize rotavirus strains. In addition, specimens were collected only during the rotavirus season, November to March. Furthermore, if MAb EIAs were incorporated in this project as the sole means to characterize rotavirus G types, 30 to 40% of specimens would have been considered nontypeable, in agreement with results from other studies (13, 24, 30; Campos et al., Abstr. 37th IDSA). RT-PCR was essential to fully characterize this collection. The practice in the past of typing only one gene product, VP7, could explain the previous failure to discover the rare strains identified here; our findings should encourage other reference laboratories to examine more than one gene product. Continued surveillance is planned to monitor changes in strain prevalence to better understand virus evolution and the shifting trends of strain patterns over time, which could affect future vaccine strategies that are predicated on the development of serotype-specific immunity to the globally common G serotypes.

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