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Serotype G9 Rotavirus Infections in Adults in Sweden

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Rotavirus is a major cause of acute gastroenteritis. By examining 1,517 stool samples collected in 2001 and 2002 from Swedish adults with acute diarrhea, rotavirus was found in 3.2%, with the emerging G9P[8] serotype being the one most commonly identified (42.9%). This is the first documentation of G9 infections in adults in Europe.

Rotavirus is the leading cause of acute viral gastroenteritis in young children worldwide and is associated with significant mortality, predominantly in developing countries. The four most common serotypes are G1 to G4, but since 1994 an increasing number of reports have demonstrated the emergence of the novel serotype G9 virus in young children (2, 5, 14).

Most recently, serotype G9 virus was also recognized as the most prevalent serotype in several parts of the world (2, 9, 15), suggesting that studies of serotype G9 should be extended. Interestingly, rotavirus infections have increasingly been recognized as causes of disease in the elderly (1, 11), but information about serotype G9 rotavirus in age groups with preexisting rotavirus immunity, such as adults and the elderly, is very limited (11) or, in the case of Europe, even lacking. We therefore addressed this question by analyzing 1,517 samples collected from Swedish adults (>15 years old) and 144 samples collected from children with acute gastroenteritis during one year (July 2001 to June 2002).

Stool analysis by electron microscopy: high number of rotavirus infections. Stool samples from throughout the country were sent to the Swedish Institute for Infectious Disease Control, Solna, Sweden, and analyzed by electron microscopy as described previously (8). In brief, a 10% fecal suspension was prepared in phosphate-buffered saline, and a drop of the suspension was incubated for 1 min on a Formvar carbon-coated grid (Agar Scientific, Stansted, United Kingdom) and then stained by 2% phosphotungstic acid (pH 6; Merck, Darmstadt, Germany) by using a "drop-on-drop" technique. To increase sensitivity, a second step was performed (centrifugation on grid), in which the fecal suspension was clarified at 20,000 × *g* for 30 min and the supernatant was pelleted directly on the grid at 150,000 × *g* for 10 min in a Beckman (Palo Alto, Calif.) Airfuge. Specimens were examined under a microscope (Philips CM 100; Eindhoven, The Netherlands) at ×46,000 magnification.

During 1 year, 1,661 stool samples from 1,517 adults (>15 years old) and 144 children with acute gastroenteritis were examined. Twenty-two percent (367 of 1,661) of the samples were found to contain virus by electron microscopy (8), and of these, 77 were rotavirus positive (21.0%). Rotavirus was identified in 50 of 1,517 (3.3%) samples collected from adults and in 27 of 144 (18.8%) samples from children.

Rotavirus G9P[8] dominates in adults. G-typing and P-type-specific PCR were performed on all rotavirus-positive samples by using previously described primers (6, 7). After amplification of segment 9 (7), the full-length VP7 genes encoding the major neutralizing outer capsid protein determining the G-type specificity were analyzed by a G1- to G4-specific reverse line blot hybridization macroassay modified from Vinje and Koopmans (16) or directly sequenced (n = 23) by using the outer primers Beg9 and End9 and the BigDye Terminator cycle sequencing kit (Perkin-Elmer). The combined data revealed that 26 of 27 samples from children could be G-typed (Table 1), with one sample being nontypeable and one sample being a mixture of G1 and G4. Among the adult samples, 49 of 50 could be G-typed, with one sample being nontypeable (Table 1). Interestingly, 42.9% (21 of 49) of the rotavirus strains collected from adults with acute gastroenteritis were serotype G9P[8]. No G9P[6] strains were identified. The second and third most prevalent types were serotype 1, identified in 30.6% of the cases, and G4, identified in 26.5% of cases (Table 1). The most common G-type in children was G1, representing 72% of the cases. While G3 strains were absent in this study, G9P[8] was identified for the first time in Sweden and repre-

TABLE 1. Rotavirus genotypes found among children and adults in Sweden from 2001 to 2002

Genotype	Children[a] (n = 25) (%)	Adults[b] (n = 49) (%)	Total (n = 74) (%)
G1	18 (72)	15 (30.6)	33 (44.6)
G2	1 (4)	0	1 (1.4)
G3	0 (0)	0 (0)	0 (0)
G4	1 (4)	13 (26.5)	14 (18.9)
G9	5 (20)	21 (42.9)	26 (35.1)

[a] These results exclude one mixed infection (G1 and G4) and one nontypeable sample.
[b] These results exclude one nontypeable sample.

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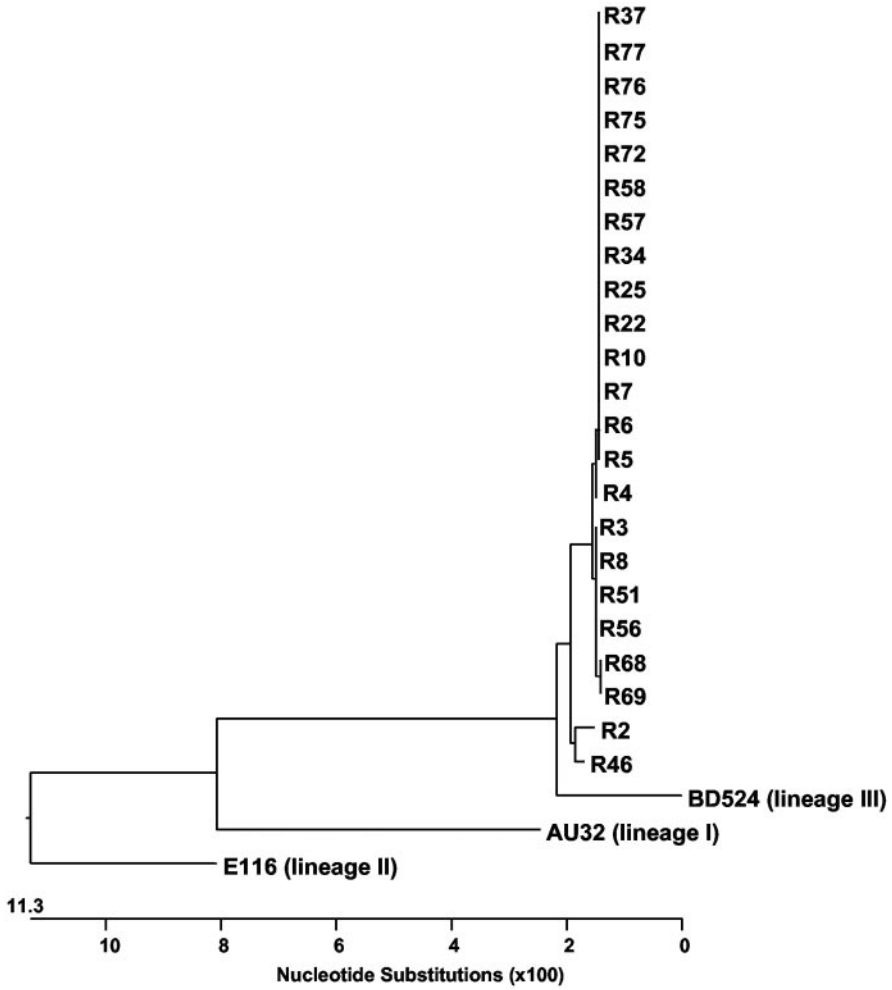

FIG. 1. Phylogenetic tree of G9 rotaviruses. Shown is a comparison of the complete coding sequences of the VP7 gene of 23 Swedish strains and three reference strains (AU32, accession number AB045372; 116E, accession number L14072; and BD524, accession number AJ250543) by the ClustalW algorithm of the MegAlign program in the DNASTAR software package (Madison, Wis.).

sented 20% of all G-typed strains from children (Table 1), excluding a mixed isolate. The P-type, determined by P[6]- and P[8]-specific reverse transcription-PCR (6, 7), of one isolate could not be identified. Altogether, G9 strains were the second most common serotype identified, representing 34.7% (26 of 75) of all typed strains from children and adults. Most of the G9 and G4 infections were found in very old patients (median age, 85 and 84 years, respectively), compared to G1 infections, which were found mainly in children (median age, 5 years). It is interesting that G9 infections were more common in adults and the elderly, an age group with preexisting rotavirus immunity, than in young children, an age group presumably without previous exposure to rotavirus. G9 viruses were identified during the entire rotavirus season (December to June). Furthermore, the isolates came from four different counties in the southern part of Sweden, suggesting that the isolates were not derived from a single sporadic outbreak.

High sequence homology among G9 strains. Sequence analysis of the full-length VP7 genes of the Swedish G9 strains revealed very high homologies, ranging between 99 and 100% identity both at the nucleotide and at the deduced amino acid levels. Comparison of the 23 Swedish sequences with a large collection of known full-length VP7 gene sequences (12) unambiguously identified the viruses as serotype G9 (data not shown). Identities with the prototype strains AU32, 116E, and BD524, representing lineages I, II, and III, respectively, were high and ranged from 88.1 to 97.7%. Phylogenetic analysis showed that all Swedish strains were more closely related to strain BD524 (96.9 to 97.7% identity) than to AU32 (88.8 to 89.2%) or 116E (88.1 to 88.4%) (Fig. 1), further confirming recent observations that all current G9 strains belong to lineage III, whereas lineage I and II consist of historical G9 strains (10).

Emergence of G9 among adults in Sweden. This study concerns G9 strains of rotavirus in adults and the elderly in Sweden, an observation not previously reported. More important, the G9 strains were even the most prevalent type, found in 43% of rotavirus-positive samples from adults, a rate higher than that previously reported for children (2, 9, 14). The fact that samples were collected from both children and adults during the same time period and geographic area and that both age groups had symptomatic G9 infections suggests that these

infections occurred independently of preexisting immunity. In order to rule out that our observations were an isolated event like the appearance of G9 strains dominating during one rotavirus season in the United States (3), we randomly selected 10 out of 66 rotavirus-positive samples from the 1999 season and sequenced their complete VP7 genes. Phylogenetic comparison (data not shown) demonstrated that two out of four samples from children and three out of six samples from adults were G9. In agreement with others (4, 13), we suggest that this result should have implications for vaccine development strategies currently targeting only serotypes G1 to G4.

Nucleotide sequence accession numbers. The reported nucleotide sequences have been given the following GenBank accession numbers: AY196111 to AY196116, AY196119, AY196121 to AY196129, and AY253833 to AY253839.

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