JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1305–1306 Vol. 38, No. 3
0095-1137/00/$04.00+0

Fast-Track Communication

Emergence of Serotype G9 Human Rotaviruses in Australia

Received 9 November 1999/Returned for modification 26 November 1999/Accepted 17 December 1999

Rotaviruses are the major cause of severe gastroenteritis in young children worldwide. The first licensed human rotavirus vaccine, the rhesus rotavirus-tetravalent vaccine (RRV-TV), incorporated serotype G1, G2, G3, and G4 specificities, as these have been the four most common serotypes causing severe disease in children globally over the last 20 years (7). The RRV-TV has recently been suspended from routine use because of an association between vaccination and increased rates of intussusception among vaccine recipients (1). Recent second-generation (bovine virus-based) vaccines also include G1 to G4 specificities (2). However, the increase in reports of other rotavirus serotypes as a cause of severe diarrhea suggests that future vaccines may need to incorporate additional specificities. Serotype G9 rotaviruses have recently emerged as important human pathogens in India (9), the United States (8), Bangladesh (10), Malawi (3), the United Kingdom (M. Iturriza, J. Green, M. Ramsay, D. Brown, U. Desselberger, and J. J. Gray, Abstr. 18th Ann. Meet. Am. Soc. Virol., abstr. W43-2, p. 136, 1999; A. D. Steele and W. D. Cubitt, Abstr. 18th Ann. Meet. Am. Soc. Virol, abstr. W43-3, p. 136, 1999), and Nigeria (V. Akran, A. Mbida, J. Mwenda, J. Muyanga, L. Nimzing, J. Nyangao, G. Pennap, D. Steele, A. Trablesi, and S. Tswana, Abstr. XIth Int. Congr. Virol., abstr. VP25.11, p. 374, 1999). This has raised interest in the global distribution of this serotype in the context of the development of effective vaccine formulations.

As part of a national surveillance program, rotavirus-positive fecal samples collected from children under 5 years of age admitted to the New Children's Hospital, Sydney, Australia, between June and August 1999 were transported to our laboratory. Routine serotyping was carried out by enzyme immunoassay (EIA) using neutralizing monoclonal antibodies (NMAbs) specific for the common serotypes G1 to G4. Thirteen of 42 samples were found to be nonreactive to these NMAbs.

Rotavirus RNA was purified from feces and subjected to reverse transcription (RT)-PCR genotyping (5) incorporating primers specific for the common serotypes G1 to G4 and the less-common human serotypes G8 and G9. Eleven samples (i.e., 26.2% of the 42 tested) yielded a 304-bp product specific for serotype G9 (Table 1). A partial sequence of the VP7 gene (which determines G serotype specificity) was determined for one of the strains (E901 [see below]), and the sequence was used to search the GenBank database for similar sequences. The best match was with the prototype G9 Indian strain, 116E, which exhibited 84.5% nucleotide identity over a 97-bp region. This analysis confirmed the G9 serotype assignment obtained by PCR. All G9 samples exhibited VP6 subgroup II specificity.

Analysis of genomic RNA segments by polyacrylamide gel electrophoresis showed that all samples displayed a "long" RNA migration pattern (electropherotype). However, three distinct electropherotypes were identified: nine isolates displayed an identical pattern (designated E901), while two isolates showed unique patterns (designated E902 and E903) characterized by mobility shifts in RNA segments 5, 7, 8, 9, and 10 (Table 1). Subsequent testing, by RT-PCR, of nonreactive (with respect to serotypes G1 to G4) samples collected from children admitted to the Royal Brisbane Hospital Australia detected one isolate (from July 1999) with G9 specificity and an E901 electropherotype (Table 1). Seven nonreactive samples collected from children admitted to the Royal Children's Hospital, Melbourne, Australia, between June and August 1999 were also typed as G9 by RT-PCR. These samples, which constituted 8.1% of samples collected during this 3-month period, also displayed an E901 electropherotype (Table 1). This demonstrated the dissemination of the E901 strain to these major Australian cities, each separated by at least 600 miles (>700 km). Serotype G9 viruses were not detected among nonreactive samples collected in other Australian cities (Adelaide and Perth) during the same period.

The antigenic properties of the three G9 strains were tested by determining their reactivities with the G9-specific NMAb F45:8 (6). The isolate displaying the E902 electropherotype reacted with F45:8, while variable reactivity was noted for E901. This suggested that the G9 strains exhibited antigenic as well as genetic variation. All strains were tested for their VP4 (P-type) specificity by nested PCR incorporating P-type-specific primers (4) and were found to belong to genotype P[8]. Thus, the features of the Australian G9 strains (subgroup II, long electropherotype, P[8]) were reminiscent of recently described human G9 viruses in the United States and Bangladesh.

Preliminary analysis (using Northern hybridization, EIA, and RT-PCR) of nonreactive rotavirus samples from stored collections has identified G9 viruses in specimens collected in Melbourne (one isolate) and Perth (two isolates) in 1997. Electrophoretic analysis indicated that these strains were different from those detected in 1999. Nevertheless, this suggests that G9 viruses had been circulating, albeit in low numbers, in the Australian community prior to 1999, when they emerged as important causes of severe diarrhea.

In summary, we have detected the first serotype G9 Australian rotavirus isolates and extended the global distribution of this emerging serotype. Analysis of genomic RNA profiles suggested that genetic diversity in circulating strains was evident. Variation in the reactivities of these strains to a G9-specific NMAb also suggested the existence of antigenic diversity. Further studies of historical collections will help to determine when these viruses first appeared in Australia. Molecular studies will determine the extent of genetic variation and the relatedness of these strains to other G9 viruses. Results here and elsewhere will have implications for rotavirus vaccine development. The incorporation of appropriate G (e.g., G9) and P (e.g., P[8]) specificities into candidate human vaccines needs to be considered.

This study was supported by Commonwealth Department of Health and Aged Care and the Royal Children's Hospital Research Institute.

TABLE 1. Serotype G9 rotaviruses isolated in Sydney, Melbourne, and Brisbane, Australia, from June to August 1999

Location	No. (%) of specimens examined that were[a]:		Strain[c]		
	Nontypeable	Serotype G9[b]	E901	E902	E903
Sydney	13 (31.0)	11 (26.2)	9	1	1
Melbourne	7 (8.1)	7 (8.1)	7	0	0
Brisbane	6 (16.7)	1 (2.7)	1	0	0
Total	26 (15.9)	19 (11.6)	17	1	1

[a] Numbers in parentheses indicate the percentages of the total number of isolates investigated during the 3-month period, i.e., for Sydney $n = 42$, for Melbourne $n = 86$, and for Brisbane $n = 36$.
[b] Determined by production of G9-specific amplicon in RT-PCR.
[c] Strain designation was based on electrophoretic migration patterns.

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