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Serotype Variation of Group A Rotaviruses over Nine Winter Epidemics in Southeastern New England

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By the use of enzyme immunoassay and RNA electrophoresis, rotavirus serotyping was performed with stool specimens from 605 children. Serotypes 1, 2, 3, and 4 accounted for 63, 9, 9, and 19% of the cases, respectively, with considerable yearly variation. An average of three serotypes cocirculated each year.

Ten rotavirus serotypes, defined by the outer capsid protein VP7, have been identified, with serotypes 1, 2, 3, and 4 being important in clinical disease (14). Protection against rotavirus infection is thought to be serotype specific and dependent on the level of neutralizing antibody against homotypic virus (2). Information on serotype distribution is important in considering the need for the inclusion of multiple serotypes in candidate vaccines. We report the results of our survey of group A rotavirus infections in southeastern New England over nine winter epidemics (1983 to 1991) to determine the prevalence and distribution of the four clinically important serotypes in the region.

(This work was presented in abstract form at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 29 September to 2 October 1991.)

Stool specimens were prospectively collected from (i) patients with diarrhea, admitted to an infant and young toddler ward at Rhode Island Hospital (RIH), and (ii) patients referred to the Diagnostic Virology Laboratory at RIH from surrounding local hospitals. Rotavirus infection was diagnosed by enzyme immunoassay (EIA) (Rotaclone, Cambridge Biotech), and the specimens were stored at −20 or −70°C until serotyped.

Rotavirus serotyping was performed by EIA with two sets of monoclonal antibodies. Initial testing was done with monoclonal antibodies provided by Tomoko Urasawa (Sapporo, Japan) and raised against strains KU (serotype 1), S2 (serotype 2), YO (serotype 3), and ST-3 (serotype 4), as previously described (12). Supplementary testing was done with a second set of monoclonal antibodies provided by Silenus Laboratories Pty, Ltd. (Victoria, Australia) and raised against strains RV-4 (serotype 1), RV-5 (serotype 2), RV-3 (serotype 3), and ST-3 (serotype 4). These antibodies were used in an indirect sandwich EIA as described by Coulson et al. (3).

Viral RNA was extracted from the stool specimens with phenol-chloroform, and electrophoresis was performed as previously described (4). The system proposed by Lourenco et al. (9) was used to assign an electrophoretic pattern. Specimens which could not be typed by EIA but which had an electrophoretic pattern identical to that of a specimen of known serotype (within the same year) were assumed to be of the same serotype.

Specimens were collected from 605 patients: 320 patients admitted to the infant and young toddler ward at RIH, 76 patients from other locations at RIH, and 209 patients seen at referring hospitals. Most were inpatients (370 of 396 RIH patients).

The Japanese monoclonal antibodies were used to test 594 specimens; a serotype was assigned to 304 specimens (51%). Serotyping on 260 specimens was done with the Australian monoclonal antibodies; 9 of the specimens were not tested initially and 251 were tested but found to be untypeable by the Japanese monoclonal antibodies, allowing serotyping of 133 (51%). The combined use of the two sets of monoclonal antibodies serotyped 438 (73%) of the 603 specimens. RNA electrophoresis was successful in 477 (79%) of 603 specimens. In 120 cases, electropherotyping was the only means of assigning a serotype. Overall, 558 (92%) of the specimens were serotyped by the combined use of EIA and RNA electrophoresis.

Figure 1 and Table 1 show the distribution of the four human serotypes. The distribution was similar between the RIH specimens and the specimens from referring hospitals and between inpatients and outpatients; therefore, the combined results are presented. Each season extended from October of a given year to September of the next year, with the vast majority of infections occurring during the months of January through April.

All serotypes were represented. Serotype 1 accounted for 349 (62.5%) of the typeable cases, with serotypes 2, 3, and 4 present in 52 (9.3%), 52 (9.3%), and 105 (18.8%) cases, respectively. Serotype 1 was present in all 9 years in proportions ranging from 18 to 94% and was the most prevalent serotype in 7 of the 9 years. Serotypes 3 and 4 were dominant in 1 year each. An average of three serotypes cocirculated every year. Serotype 2 was sporadically present during most years without being predominant. Serotype 3 appeared during the 1986–1987 season, became predominant during the 1987–1988 season, and then decreased markedly. Serotype 4 was dominant during the 1984–1985 season and was uncommon thereafter.

The rotaviruses grouped into 14 different electrophoretic patterns. Serotype 1 had seven different patterns, serotypes 2 and 3 had three each, and serotype 4 had four. Except for the first year, each year had at least four and as many as seven different cocirculating strains. A common occurrence was the presence of one predominant electropherotype at the beginning of the season followed by the emergence of several different electropherotypes at the end of the season. Serotype 1 showed the most variability, with the "caha"

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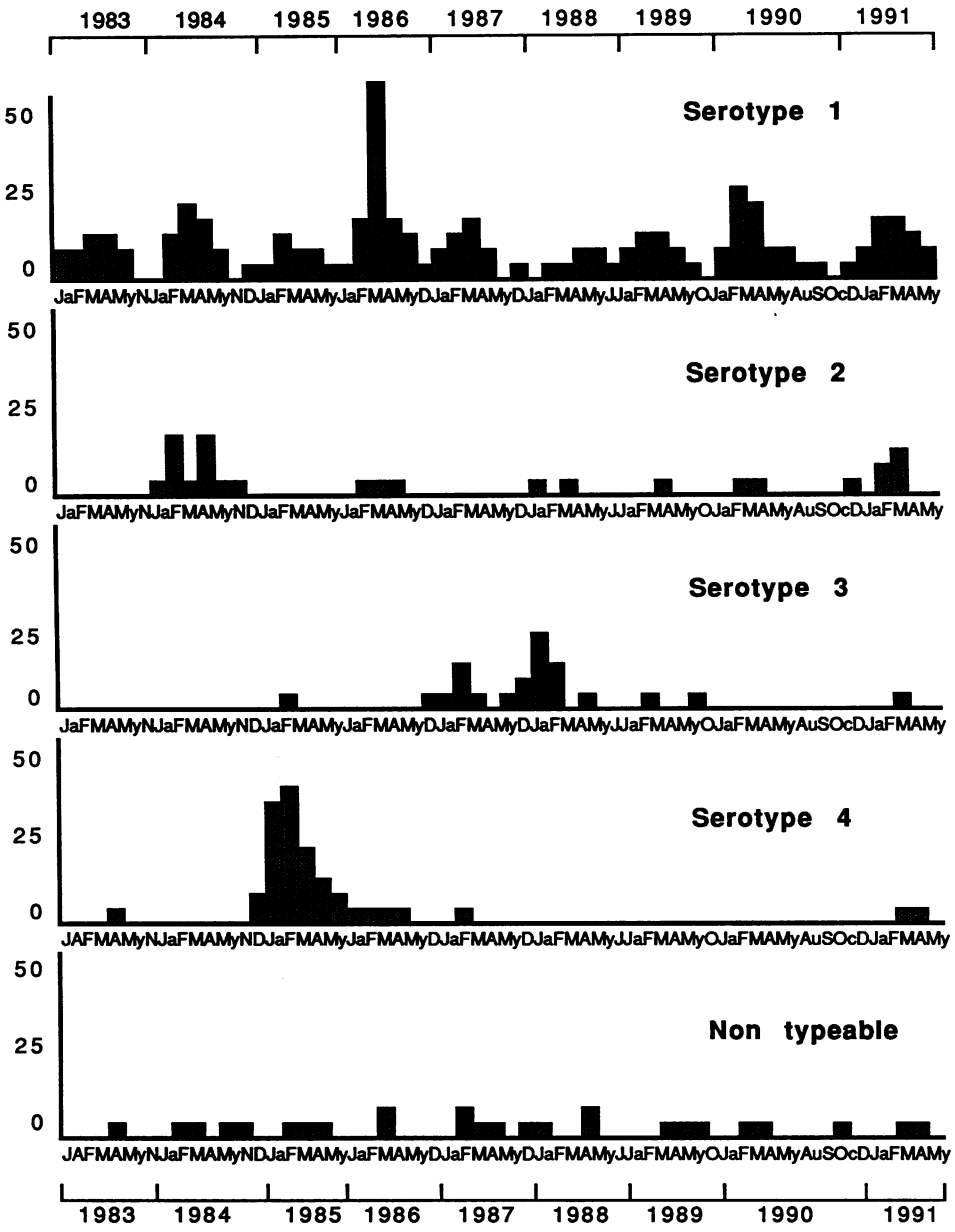

FIG. 1. Distribution (number of cases) of group A rotavirus infections in southeastern New England, 1983 to 1991.

strain of serotype 1 present over most of the study period. Serotype 2 always demonstrated a "short" migration pattern, as has been previously described. Serotype 3 had a characteristic pattern in the third migration zone, designated "i," consisting of a thin band followed by a thick band. Serotype 4 had patterns resembling those of either serotype 1 or 3 but occurring in a different season. A specific pattern corresponded to the same serotype during a particular season but could correspond to a different serotype in another season. Representative electropherotypes are shown in Fig. 2.

The present study demonstrates a marked year-to-year variation in the distribution of the four human rotavirus serotypes, 1, 2, 3, and 4. Similar findings have been reported by other investigators in Texas and the north-central United States (10), Australia (1), and Venezuela (13). Recognition of

TABLE 1. Yearly distribution of group A rotavirus serotypes in southeastern New England

Season	No. of specimens[a]					
	Total	st 1	st 2	st 3	st 4	NT
1982–1983	35	**33**			1	1
1983–1984	77	**42**	28			7
1984–1985	119	21		1	**92**	5
1985–1986	92	**80**	4		5	3
1986–1987	60	**32**		17	2	9
1987–1988	52	15	2	**29**		6
1988–1989	43	**36**	1	1		5
1989–1990	62	**50**	4	3		5
1990–1991	65	**40**	13	1	5	6
Total	605	349	52	52	105	47

[a] The predominant serotype (st) for each season is in boldface. NT, nontypeable.

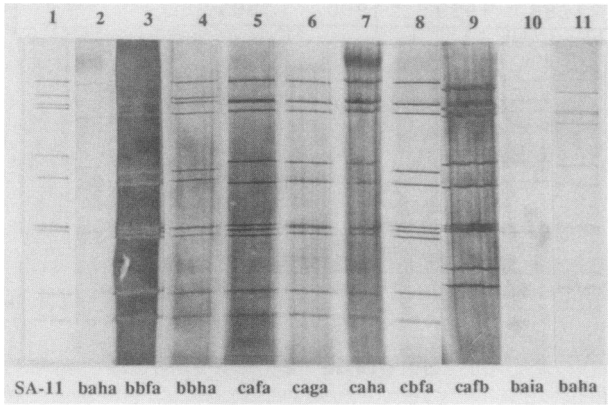

FIG. 2. Examples of electrophoretic patterns. Lanes: 1, SA-11 (standard for comparison); 2 to 8, serotype 1; 9, serotype 2; 10, serotype 3; 11, serotype 4.

this variability is important, since it may explain the conflicting results in vaccine efficacy trials and may also affect the development of vaccine strategies (5, 8). Multiple candidate vaccines are currently being developed in order to prevent severe rotavirus disease. These include reassortment vaccines which express the outer capsid polypeptide VP7 of the four important human serotypes. The data from studies of serotype variability suggest that in order to be highly effective, a candidate vaccine should provide immunity against the four serotypes.

The use of EIA allowed the screening of a large number of specimens, which would have been impossible to achieve by using the more traditional neutralization assays. Each set of monoclonal antibodies could assign a serotype in 51% of the specimens. The study was not designed to compare the two sets, and they were not necessarily used with the same specimens. Some specimens could not be typed by EIA; this phenomenon was most likely related to denaturation of the VP7 as a result of repeated freezing and thawing of the specimen or to an epitope on the VP7 which might not have matched that recognized by the specific monoclonal antibody. Accumulating evidence indicates a strong association between the electrophoretic pattern and the corresponding serotype, so that each RNA pattern corresponds to one serotype within the same season and in a confined geographical area (7, 10). RNA electropherotyping, even though more laborious, proved very helpful in delineating the serotypes in the specimens not typeable by EIA. Only 47 specimens (7.8%) could not be assigned a serotype after the combined use of EIA and RNA electropherotyping. Electropherotyping provided further evidence of the variability of the infecting strains in our population. Of note, the RNA electrophoretic patterns of serotype 4 resembled those of serotype 1 in many cases. The protein products are likely to be similar in structure and possibly immunogenicity, which is consistent with the fact that a heterotypic immune response has been noted between these two serotypes (6, 11).

In summary, the results of the present study demonstrate that EIA serotyping with anti-VP7-specific monoclonal antibodies and RNA electropherotyping are useful tools to assess the serotypic variation of rotavirus causing infection in a community. The marked year-to-year variability suggests that a vaccine strategy against rotavirus in our community is more likely to succeed if protection against all the four serotypes is provided.

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