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Temporal and Geographical Distributions of Human Rotavirus Serotypes, 1983 to 1988

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Between 1983 and 1988, subgroups and serotypes were determined for 907 of 1,084 clinical specimens of rotaviruses collected in various countries of Europe, North and South America, Africa, and Asia. Enhanced enzyme immunoassays based on monoclonal antibodies specific for rotavirus proteins VP6 and VP7 were used. Significant differences in the prevalent serotypes were detected from year to year in the United Kingdom and Brazil and also in different countries during the same year. Throughout the study, rotavirus serotype 1 was detected most often (53.8%), followed in frequency by serotype 2 (17.8%), serotype 3 (12.1%), serotype 4 (11.1%), and serotypes other than 1 to 4 (5.1%). No individual serotype was found to predominate consistently in any one location. In the United Kingdom, rotavirus serotypes varied in prevalence in a regular but not predictable way. We suggest that a similar epidemiology might be found in other settings. Seventeen unusual strains were detected. Of these, five strains did not react with reference monoclonal antibodies specific for subgroup I and subgroup II, but they reacted with rotavirus group A-specific polyclonal and monoclonal antibodies; four strains were of subgroup II, serotype 2, and at least one had a "long" electropherotype; two strains were of subgroup I, serotype 2 with a long electropherotype; and one strain was of subgroup I, serotype 3. Five group C rotaviruses were detected.

Rotaviruses are now well established as the most frequent viral pathogen detected in cases of acute gastroenteritis in children under 2 years of age throughout the world (20–22). In developed countries, rotaviruses are responsible for 40 to 60% of cases of severe dehydrating diarrhea in children under 2 years of age who require hospitalization, although deaths are rare. In the developing world, rotavirus is an equally important pathogen, and deaths from dehydration are common (20).

According to figures produced by the World Health Organization (WHO), an effective vaccine against rotavirus diarrhea could reduce deaths by up to 30%, thus averting between 200,000 to 300,000 deaths in children under 2 years of age annually (20–22).

A number of candidate vaccines have been developed and tested (3, 13, 35, 36, 53–55). The degree of protection afforded by each vaccine has varied greatly (53). The RIT 4237 vaccine, a candidate rotavirus vaccine derived from a bovine strain of serotype 6 (54), protected children against severe infection with rotavirus serotype 1 in Finland (54) but failed to afford any protection in Rwanda and Peru (53), where rotavirus serotypes other than type 1 were shown to be causing infections. Promising results have been obtained with a candidate vaccine derived from a serotype 3 rhesus rotavirus strain, the MMU-17706 vaccine, especially in outbreaks involving serotype 3 rotaviruses (3).

It is thought that one of the factors affecting the efficacy of candidate vaccines is the serotype or serotypes of rotaviruses circulating naturally in the communities under investigation (53). Although several reports on the epidemiology of rotavirus serotypes in different settings have appeared in recent years (8, 9, 12, 26, 29, 33, 37, 38, 42, 47, 51, 52), little is known about differences in their distribution between countries during the same year and within the same country from year to year (see Discussion).

Rotaviruses have at least two surface antigens involved in neutralization which are of importance in protective immunity. They are designated VP4 and VP7 (39, 45). The classification of rotavirus serotypes is at present based exclusively on the VP7 antigen (2, 7, 8, 34, 56, 57), although the importance of the role of VP4 in both immunity and pathogenicity is emerging (18, 33, 44, 45). Although neutralizing monoclonal antibodies to VP4 are becoming available, few are characterized fully, and the epidemiology of rotaviruses based on their VP4 specificities remains to be investigated.

In this report, we present the results of a retrospective study of the distribution and epidemiology of rotavirus serotypes (defined by serotype-specific neutralizing monoclonal antibodies to VP7) in various countries of Europe, North and South America, Africa, and Asia between 1983 and 1988.

MATERIALS AND METHODS

Rotaviruses. One thousand and eighty-four fecal samples from children with acute gastroenteritis were tested for rotavirus by the WHO enzyme-linked immunosorbent assay (ELISA) for rotavirus antigen (6).

Primary testing was done in WHO-sponsored laboratories in South America, Africa, and Asia (Table 1). Rotavirus-positive samples were then sent to our laboratory for further characterization. Samples from other laboratories in North America and Europe which were originally tested by other methods were also sent to our laboratory, where the presence of rotavirus was confirmed by the WHO assay (Table 1). The United Kingdom specimens were almost entirely from the West Midlands region. Samples were received as either undiluted feces or as approximately 10 to 20% (vol/vol) extracts in either phosphate-buffered saline (PBS), pH 7.2, or 0.1 M Tris hydrochloride, pH 7. Samples were stored at −70°C before testing.

Antisera and monoclonal antibodies. Polyclonal antisera to rotavirus were produced in rabbits and a guinea pig as described previously (6). Rabbit antisera were used as cap-

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TABLE 1. Temporal and geographical distributions of human rotavirus serotypes from 1983 to 1988

Yr	Country of Origin	Total no. of specimens serotyped[a]	No. of specimens of serotype:					Source or reference
			1	2	3	4	n[b]	
1983	United Kingdom	87	42	12	21	10	2	Unpublished data
	Sweden	32	16	11	5	0	0	Unpublished data
	Peru	54	10	31	3	6	4	Unpublished data
1984	United Kingdom	33	14	11	5	0	3	23 and unpublished data
	Finland	42	36	2	4	0	0	54
	Canada	41	23	4	11	0	3	Unpublished data
	Brazil	22	8	7	2	2	3	37 and unpublished data
	Central African Republic	152	102	22	19	3	6	26
	Indian (Tamil Nadu state)	46	15	3	2	12	14	12
1985	United Kingdom	22	20	2	0	0	0	Unpublished data
	Brazil	35	7	4	3	19	2	37 and unpublished data
	Gambia	3	1	1	0	0	1	47
	Pakistan	12	0	0	0	12	0	Unpublished data
1986	United Kingdom	84	40	7	14	21	2	Unpublished data
	Finland	64	56	2	0	3	3	53
	Burma	17	4	3	6	3	1	Unpublished data
1987	United Kingdom	76	52	9	13	2	0	Unpublished data
	Finland	29	21	2	0	3	3	53
	Sri Lanka	5	0	0	0	5	0	Unpublished data
1988	United Kingdom	51	21	28	2	0	0	Unpublished data

[a] Of 1,084 specimens tested from 1983 to 1988, 177 were either negative for VP7 or low in antigen.
[b] n, Positive for VP7 but not for serotypes 1 to 4.

ture antibodies in the group-, subgroup-, and serotype-specific ELISAs (5, 6). The guinea pig antiserum was used as the detector antibody in the WHO ELISA for group A rotavirus antigen. All polyclonal antisera were diluted 1/10,000 before use as described previously (5, 6).

Eight different monoclonal antibodies were used; they were A3M4, SGI, SGII, 60, RV4:2, RV5:3, RV3:1, and ST:3. Monoclonal antibody A3M4 is specific for rotavirus group A and was produced in our laboratory (6); it reacts with VP6 of both subgroup I and subgroup II rotaviruses on Western (immuno-) blots (unpublished data). Monoclonal antibodies 255/60 and 631/9 are subgroup specific (against subgroups I and II, respectively). Monoclonal antibody 60 is cross-reactive with VP7 of different serotypes (e.g., serotypes, 1, 2, 3, 4, 6, and 8) and was used as a control to indicate that complete particles were present. Monoclonal antibodies 255/60, 631/9, and 60 were generous gifts from H. B. Greenberg and R. D. Shaw (49). Monoclonal antibodies RV4:2, RV5:3, RV3:1, and ST:3 are specific for the VP7 of rotavirus serotypes 1 to 4, respectively; these were generous gifts from B. Coulson (15–17). All monoclonal antibodies as ascitic fluids were diluted 1/10,000 as described previously (5–7).

Two enzyme-conjugated antibodies were obtained from commercial sources. (i) An affinity-purified antibody to total guinea pig immunoglobulin G produced in goat and labeled with alkaline phosphatase from calf intestine. This was obtained from Kirkegaard and Perry Laboratories, Gaithersburg, Md., and was used in the WHO rotavirus group A ELISA at a 1/500 dilution as described previously (6). (ii) For the subgroup- and serotype-specific ELISAs, an affinity-isolated, anti-murine polyvalent immunoglobulin antiserum, also produced in a goat and labeled with calf intestinal alkaline phosphatase, was used at a 1/1,000 dilution as described previously (7). This was obtained from Sigma Chemical Co., St. Louis, Mo.

WHO indirect ELISA for group A rotavirus antigen. The WHO indirect ELISA for group A rotavirus antigen was developed in this laboratory and dispatched to WHO-sponsored laboratories in the form of a kit containing most reagents in a lyophilized form. The test was a double-antibody sandwich ELISA using polyclonal, polyvalent hyperimmune rabbit and guinea pig anti-rotavirus sera. A full description of the assay has been published previously (6). Briefly, 100 μl of a hyperimmune rabbit antiserum to rotavirus diluted 1/10,000 in 0.1 M sodium carbonate–sodium hydrogen carbonate buffer, pH 9.8 was adsorbed to the wells of polystyrene microdilution plates. The test samples were diluted 1/4 in PBS containing 0.1% polyoxyethylene sorbitan monolaurate (Tween 20) and 0.01 M EDTA disodium salt (PBS-Tween 20). A 100-μl portion of sample was added to duplicate wells. The plates were held at 4°C overnight and washed six times with PBS-Tween 20, and 100 μl of a hyperimmune guinea pig antiserum to rotavirus diluted 1/10,000 in PBS-Tween 20 containing 1% bovine serum albumin (BSA) was added to each well. After incubation at 37°C for 2 h, the plates were washed six times in PBS-Tween 20, and 100 μl of goat anti-guinea pig immunoglobulin G-alkaline phosphatase conjugate diluted 1/500 in PBS-Tween 20-BSA was added to each well. After incubation for 1 h at 37°C, the plates were washed as described above and 100 μl of substrate (*p*-nitrophenyl phosphate disodium, 1 mg/ml in 0.1 M diethanolamine buffer, pH 9.8) was added to each well. The reactions were stopped after 20 min by the addition of 50 μl of 3 M NaOH to each well. Optical densities were measured for each well at a wavelength of 405 nm. Samples generating optical density readings of >0.1 were confirmed

as positive by a competitive assay in which a competing antibody was included in the sample diluent.

Enhanced enzyme immunoassays for subgroup and serotype determination of rotaviruses. Serotyping and subgrouping of rotavirus-positive samples were performed by using highly sensitive enhanced ELISAs based on subgroup-specific and serotype-specific monoclonal antibodies. The tests have been described in detail previously (5). Briefly, polystyrene microdilution plates were coated with 100 μl of a 1/10,000 dilution of serum from a rabbit which had been hyperimmunized with a mixture of rotavirus isolates representing serotypes 1 to 4. The plates were held at 4°C overnight, and then the wells were emptied. Stool samples (100 μl of 10 to 20% [vol/vol] extracts in 0.1 M Tris-buffered saline [TBS], pH 7.5, containing 0.1% [vol/vol] Tween 20 and 3% [wt/vol] BSA) were added to each of 16 wells in pairs across the plate. The plates were kept at 4°C overnight and then washed six times with TBS-Tween 20. Monoclonal antibodies in the form of ascitic fluids were diluted 1/10,000 in TBS-Tween 20-BSA, and 100 μl was added to two wells for each antibody. The plates were incubated for 2 h at 37°C and then washed.

Goat anti-murine polyvalent gamma globulin-alkaline phosphatase conjugate was diluted 1/1,000 in TBS-Tween 20-BSA, and 100 μl was added to each well. The plates were incubated at 37°C for 1.5 h and washed six times with TBS-Tween 20.

NADP-substrate (100 μl; IQ Bio Ltd., Cambridge, United Kingdom) was added to each well, and the plates were left at room temperature (approximately 22 to 24°C) for 15 min. The plates were not washed. Ethanol-INT violet amplifier solution (200 μl; IQ Bio) was added to the 100 μl of substrate. The reaction was stopped with 3 M sulfuric acid after 15 min. Optical densities were read at 492 nm.

Samples were considered to give a positive result with any serotype-specific monoclonal antibody if the optical density obtained was at least 2.5 times the value of the average optical densities obtained with the other antibodies if these were <0.1.

Polyacrylamide gel electrophoresis of rotavirus genomic RNAs. For RNA extraction and electrophoresis the methods described by Rodger et al. (46) were used, and the RNAs were visualized either by the silver-staining method reported by Herring et al. (30), or by staining with ethidium bromide (1 mg/ml in distilled water) (46).

Statistical analysis. Differences in the prevalence of rotavirus serotypes in the same settings in different years and in different settings during the same year were tested for significance by using the chi-square test on absolute values. Yates' correction for low numbers was used throughout. In cases in which a serotype was not detected, an arbitrary value of 1 for the absent serotype was entered in the calculations.

RESULTS

The results of some of the tests have been published previously (Table 1). All of the serotype and subgroup results listed in Table 1 were determined in our laboratory.

Subgroup determination. With only a few exceptions (see below), all serotype 2 rotaviruses (161; Table 1) were of subgroup I, and all rotaviruses of serotypes 1, 3, and 4 (699; Table 1) were of subgroup II. Of the 47 samples which could not be serotyped but were positive for VP7 (see below), 36 were of subgroup II and 11 were of subgroup I. Of the 177 samples that were negative for VP7, 71 were of subgroup I and 101 were of subgroup II. Five specimens did not react with either of the subgroup-specific monoclonal antibodies but did react with rotavirus group A-specific polyclonal and monoclonal antibodies.

Distribution of rotavirus serotypes by year and country, 1983 to 1988. In Table 1, the serotypes of all the VP7-positive samples tested are presented according to year and location. In all, 907 samples of 1,084 which were positive by the WHO assay for group A antigen were also positive for VP7. Of these, 860 (94.9%) were of serotypes 1 to 4, and 47 (5.1%) were positive for VP7 but did not react with the monoclonal antibodies specific for serotypes 1 to 4. Serotypes 1 to 4 were detected throughout the world, and no individual serotype appeared to be confined to any one location. Serotype 1 rotavirus was detected most frequently (53.8%), followed in frequency by serotype 2 (17.8%), serotype 3 (12.1%), and serotype 4 (11.1%). However, there were significant differences in the distribution of rotavirus serotypes in individual countries year by year and in different countries during the same years (see below). There appeared to be no difference in the variety of serotypes found in developed and developing countries; thus, the degrees of cocirculation of different rotavirus serotypes in each setting were similar.

Distribution of rotavirus serotypes in the United Kingdom from 1983 to 1988. In the United Kingdom, as in other countries with a temperate climate, rotaviruses are at their highest prevalence during the winter months, as has been described previously (10, 23). The data shown in the serotyping results include samples collected during the later months of the previous calendar year. Thus, for example, samples dated 1984 include all samples collected between October 1983 and April 1984. (The few samples collected between May and September of each year were not included.)

The prevalence of rotavirus serotypes in the West Midlands region of the United Kingdom is illustrated in Fig. 1. When the data for 1983 were used as a baseline, at least one significant difference in the distribution of serotypes was detected in 1984, and similar differences were found for subsequent years when they were compared with the previous year. Thus, in 1984 there was a significant increase in the prevalence of serotype 2 rotaviruses; in 1985, the numbers of serotype 2 rotaviruses detected were significantly lower ($P < 0.05$), and there was a corresponding increase in the prevalence of serotype 1 rotaviruses ($P < 0.01$); in 1986, serotype 4 rotaviruses emerged as the second dominant strain ($P < 0.05$), and there was a significant reduction in the numbers of serotype 1 rotaviruses in circulation ($P < 0.01$); the situation was the reverse in 1987, when there was a significant increase in serotype 1 rotaviruses ($P < 0.05$) accompanied by a reduced prevalence of serotype 4 rotaviruses ($P < 0.01$); in 1988, serotype 2 rotaviruses appeared as the dominant strain for the first time during the study ($P < 0.01$). Serotypes 1, 2, and 3 cocirculated during most years of the study, except for 1985, when serotype 3 rotavirus was not detected; serotype 4 rotaviruses appeared intermittently in 1983, 1986, and 1987, but none was detected in 1988. In 1983, 1984, and 1986, an average of 1.6 samples were detected in each year which were positive for VP7 but did not react with monoclonal antibodies specific for serotypes 1 to 4, but on no occasion did these numbers significantly differ from one year to the next.

Distribution of rotavirus serotypes in Brazil in 1984 and 1985. Serotypes 1 to 4 and serotypes other than 1 to 4 were detected in Brazil in both 1984 and 1985 (Fig. 2). In 1985,

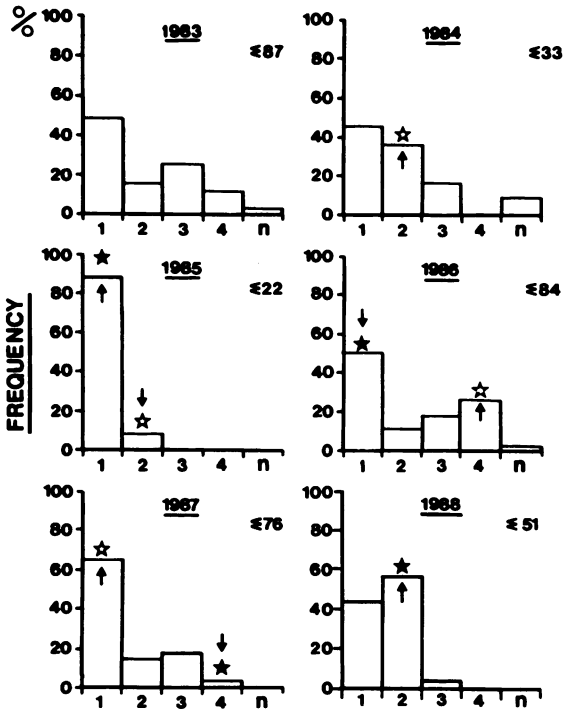

FIG. 1. Distribution of rotavirus serotypes detected in the West Midlands, United Kingdom between 1983 and 1988. The prevalence of rotavirus serotypes is shown as a percentage of the total numbers of VP7 samples tested. In all cases, chi-square tests were performed on absolute values. Significant differences are indicated (★, $P < 0.01$; ☆, $P < 0.05$). Arrows indicate significant increases (up) or decreases (down) in prevalence. 1, 2, 3, 4, and n, Serotypes 1 to 4 and not 1 to 4, respectively. The total number of specimens tested for each year is shown in the top right corner of each graph after a capital sigma (Σ).

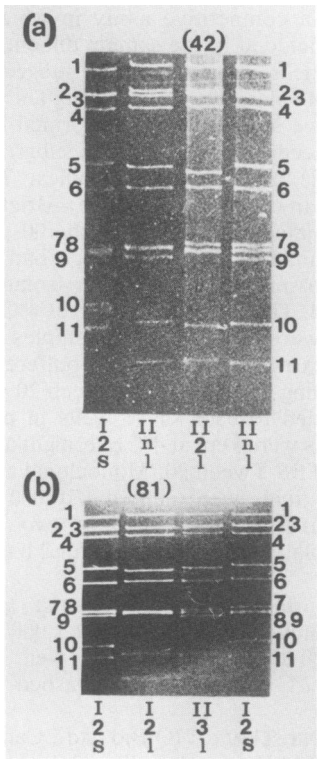

FIG. 3. Polyacrylamide gel electrophoresis of genomic RNAs of two unusual strains. (a) Sample 42; RNA of a subgroup II, serotype 2 rotavirus with a long electropherotype. (b) Sample 81; RNA of a subgroup I, serotype 2 rotavirus with a long electropherotype. Numbers at the sides denote RNA segments. Roman numerals at the bottom indicate subgroup, arabic numbers indicate serotype, and s and l refer to short and long RNA profiles, respectively.

there was a significant increase in the number of serotype 4 rotaviruses in circulation ($P < 0.01$).

Differences in the distribution of rotavirus serotypes in different countries in 1984. The distribution of rotavirus serotypes found to be circulating in 1984 in the United Kingdom, Finland, and Canada was tested for significant differences in prevalent serotypes. There were significant differences in the numbers of serotype 1 ($P < 0.01$) and 2 ($P < 0.01$) rotaviruses in Finland compared with in the United Kingdom. Similarly, there was a significant difference in the number of serotype 2 rotaviruses circulating in Canada compared with in the United Kingdom ($P < 0.01$) and in the numbers of serotype 1, 2, and 3 rotaviruses in circulation compared with in Finland ($P < 0.01$ in each case).

RNA polyacrylamide gel electrophoresis. Although not performed for all specimens, the comparison of RNA profiles with the subgroups and serotypes of isolates revealed a number of unusual strains. Four subgroup II, serotype 2 strains were detected, at least one of which had a "long" (fast-migrating segment 11) electropherotype. One subgroup I, serotype 3 strain was detected in a specimen from Brazil, and two subgroup I, serotype 2 strains with long electropherotypes were detected in Brazil and India (Fig. 3a and b). Five group C rotaviruses were detected by electron microscopy; they were negative in the group A-specific WHO assay. The RNA profiles of these isolates were similar to those of group C rotaviruses described elsewhere (11). Four of these samples were confirmed as group C rotaviruses by immune electron microscopy with a standard group C antiserum (12).

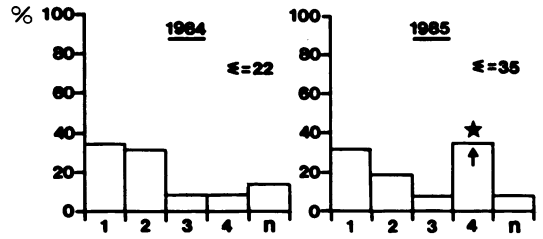

FIG. 2. Distribution of rotavirus serotypes in Brazil in 1984 and 1985. Symbols are as explained in the legend to Fig. 1.

DISCUSSION

Until recently, little was known about the distribution of rotavirus serotypes in developing countries (8, 12, 26, 29, 33, 37, 38, 42, 47). Even in developed countries, information has been limited.

Our laboratory has undertaken a retrospective study over 6 years of the distribution of rotavirus serotypes throughout various countries of the world.

A number of problems were encountered during this study. Of particular relevance was the difficulty in arranging the testing for serotype and subgroup specificities soon after the original diagnostic assays had been performed. Problems with storage and transportation from the WHO-sponsored

laboratories in developing countries to the United Kingdom resulted in a number of specimens being rendered unsuitable for serotype testing in the enhanced ELISA because of the absence of VP7. In the end, we were able to serotype 907 rotavirus samples and thus obtained some information on the temporal and geographical distribution of serotypes.

A number of previous reports on the geographical and temporal distribution of different group A rotaviruses have been published. These were based on either subgroup analysis or RNA profiles (electropherotyping) or both but not on the analysis of serotypes (1, 10 27, 46, 58). Those studies have confirmed that rotaviruses occur throughout the world and have a consistent seasonal pattern in many settings, particularly in developed countries with a temperate climate, where they have been shown to occur most frequently during the cooler months of the year (10).

The study of Brandt et al. (10) reported the distribution of rotavirus subgroups detected in Washington, D.C., between January 1974 and June 1978, representing five rotavirus seasons. Although subgroup II rotaviruses were predominant during most of the years, there were significant changes in prevalence in favor of subgroup I rotaviruses during the winter of late 1977 and early 1978. A similar finding was reported from South Africa, where between March 1983 and December 1986 subgroup II rotaviruses were the most prevalent, except during March 1984 (50). Cocirculation of serologically different rotaviruses was also a consistent finding (50).

Of the studies using RNA electrophoresis, that of Rodger et al. is one of the longest, covering 6 years (46). The authors reported a sequential pattern of appearance and disappearance of electropherotypes and cocirculation of different rotaviruses. Similar findings have been reported by others (23). However, it should be stressed that RNA electrophoresis does not reveal serological differences and can also conceal genomic differences (4, 19, 23) (see below).

Until recently, information on the distribution of rotavirus serotypes has been limited because of difficulties in propagating rotaviruses in routine cell cultures and because of the fact that serotype-specific monoclonal antibodies have not been widely available. Our laboratory has been fortunate in the latter respect, and preliminary reports on the distribution of rotavirus serotypes in India, Brazil, Africa, and other settings have been published (5, 12, 26, 37) (Table 1). Cocirculation of different rotavirus serotypes was found in all settings.

In a 2-year study on rotaviruses isolated in Bangui, Central African Republic, from January 1983 to March 1985, 143 rotaviruses were serotyped. Serotype 1 was shown to be dominant, except during October to December 1983, when there was a significant increase in the prevalence of serotype 2 rotavirus, which became the predominant strain during those months (26). Similarly, in a 3-year prospective study in Belem, Brazil, serotype 1 rotavirus was dominant during the first 18 months, but then serotype 2 rotavirus emerged as the dominant strain, although serotypes 1, 3, and 4 cocirculated (37). In 1985, there was a significant increase in the prevalence of serotype 4 rotavirus (see above). Similarly, a study of rotavirus serotypes in southern India detected an increase in serotype 4 rotavirus towards the end of the study period.

The significant change in prevalent serotype each year in the United Kingdom was expected from earlier findings (23, 48). In an analysis of outbreaks in Glasgow, Scotland, between 1981 and 1983, a significant change in serotype prevalence in favor of serotype 1 was observed in the winter of late 1982 and early 1983. Regional variations within the United Kingdom were also observed (23).

Although these periodic changes in serotype prevalence could be demonstrated in detail only in the United Kingdom, the results of the testing of samples from other countries suggest that this might be a common occurrence. Thus, in a recent report from the People's Republic of China (59), a similar change in dominant rotavirus serotype was detected each year between 1982 and 1985 in Guangzhou and Foshan provinces; in this study, a serotype-specific cDNA hybridization assay was used. All four serotypes were detected in the survey, but a single serotype was dominant in each year, with "minimal cocirculation" of other serotypes. Similarly, a predominant serotype (serotype 1) was found throughout Australia in 1986 and 1987, and other serotypes occurred infrequently in certain locations only (51). In contrast, a great fluctuation of different serotypes was recorded in Melbourne, Australia, between 1977 and 1986 (9). Testing of 562 rotavirus samples collected in Japan between November 1986 and March 1988 showed that serotypes differed in prevalence in different regions during the same year and that a "yearly change in the prevalence of individual serotypes in the same locale was noted" (52). Serotypes 1 to 4 constituted the great majority, but 7.7% of samples, although positive for VP7, could not be serotyped "and may represent new serotypes" (52). Thus, our findings are in agreement with those of several other groups in different countries.

The detection of rotaviruses with unusual subgroup, serotype, and RNA profile associations has been described previously (1, 41, 43, 51). It had been assumed initially that the correlations of (i) subgroup I and "short" RNA electropherotype with serotype 2 specificity and of (ii) subgroup II and long electropherotype with serotype 1, 3, and 4 specificities for human rotaviruses would hold when further strains were tested (7, 28). Although, for the great majority of human rotaviruses tested to date, the two main associations of subgroup, serotype, and electropherotype have been confirmed, this report and others have shown that RNA electropherotyping cannot be used reliably to predict subgroup or serotype, nor can subgrouping be used to predict serotype or electropherotype (1, 4, 23, 27, 41, 42, 50). We have included the determination of subgroups in our study in order to identify such unusual subgroup-serotype associations in our material; such strains might be of epidemiological significance as strains of animal origin in the human population.

Studies in vitro have demonstrated that all RNA segments can undergo independent genetic reassortment, that subgroup (VP6) specificity cosegregates with segment 6 only, and that serotype (VP7) specificity cosegregates with segments 7, 8, or 9 (24, 28, 31, 32, 40). This is because the proteins which specify subgroup (VP6) and serotype (VP7) are coded for by RNA segments 6 and 7, 8, or 9, respectively, whereas short and long electropherotypes are designated by differences in the relative migration of segments 10 and 11. Furthermore, RNA segments that comigrate on gels have been shown to have distinct sequences (4, 23).

A recent report has described the detection of 39 subgroup I rotaviruses with a long electropherotype among 78 samples of human rotaviruses, but the serotype specificities of these samples have not been reported (27). Two subgroup I strains with a long electropherotype have been isolated in Japan and have been shown to be of serotype 3 (41, 42). RNA-RNA hybridization studies of these strains revealed strong homologies with a serotype 3, subgroup I feline rotavirus (41).

These findings are of importance to the molecular evolu-

tion of rotaviruses (18) and suggest that, although subgroup and electropherotype are of some value to epidemiology, their use in rotavirus classification is limited and serotype specificities based on VP7 and VP4 antigens take priority (7, 8, 14, 16, 25, 29, 31, 33, 34, 40, 45, 49, 57).

It is not surprising that 5.1% of the samples could not be serotyped with monoclonal antibodies which were serotype specific for serotypes 1 to 4. The existence of at least nine serotypes of rotaviruses infecting humans and animals is now documented (7, 8, 16, 29, 32–34, 38, 44, 49, 56, 57). However, this study has shown that infections of humans by rotavirus serotypes other than 1 to 4 seem to be rare, constituting only 5% of the total.

Furthermore, given the ubiquitous distribution of rotavirus serotypes 1 to 4 throughout the world, reinfections with different rotavirus serotypes should occur with some frequency. In at least one prospective longitudinal study (37), it has been shown that 16% of the children investigated had one or two reinfections and that in some of the children "the initial infection (either symptomatic or asymptomatic) caused by serotype 1, was followed by a subsequent diarrheic episode associated with serotype 2" (37). Thus, reinfections with different serotypes occur, and the immune response to one serotype does not necessarily protect against heterotypic strains.

Overall, it appears that prevention of rotavirus disease by vaccines comprising the four serotypes causing 95% of the infections might be an achievable goal. However, several questions remain to be answered, such as the temporal and geographical distribution of rotaviruses based on VP4 specificities and the effect of interventions involving live attenuated vaccines on the epidemiology of rotavirus serotypes.

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