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# Epidemiology of Rotavirus Serotypes in Melbourne, Australia, from 1973 to 1989

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**Fecal rotavirus strains collected between 1973 and 1989 from 943 children admitted with acute diarrhea to one hospital in Melbourne, Australia, were serotyped by using an enzyme-linked immunosorbent assay. The assay incorporated neutralizing monoclonal antibodies specific for VP7 of the four major human serotypes (1 through 4). A serotype could be assigned to 690 of 943 specimens (73.2%). Typeable strains comprised serotype 1 (72.5%), serotype 2 (6.8%), serotype 3 (2.9%), or serotype 4 (15.4%). Monotypes 1a and 1c comprised 52 and 44%, respectively, of serotype 1 strains. All serotypes and monotypes exhibited polymorphic genomic RNAs. Specimens reacting as mixed serotypes were rare (3.2%) and included intertypic strains (0.7%) and mixed infections (1.0%). Nontypeable strains for which an electropherotype could be determined appeared to be identical with typeable strains present concurrently in the community. Serotypes exhibited various epidemiological patterns. Serotype 1 strains were dominant except during three successive winters when 60 to 90% of the disease was caused by serotype 2. Serotype 4 strains showed an episodic pattern of appearance, recurring at peak incidence approximately every 3 years. Fecal rotavirus strains collected from 145 newborn babies housed in Melbourne obstetric hospitals between 1974 and 1986 were also serotyped. All 135 typeable strains (93.1%) belonged to serotype 3. It is hypothesized that endemic infection with serotype 3 rotaviruses in nurseries for the newborn influenced the epidemiology of rotavirus serotypes responsible for severe clinical disease in young children in the same community.**

Throughout the world, rotavirus infection is the single most common cause of severe watery diarrhea during the first 2 years of life (5). Hospital-based studies have shown that rotavirus disease is endemic in tropical countries and that annual epidemics of rotavirus infection occur during the colder months in temperate climates. Analysis of genomic RNA electropherotypes of rotaviruses has shown great genetic diversity in strains, even in those simultaneously present in the same geographical location (15, 29). In most localities, there is a sequential pattern of appearance of electropherotypes, with predominant strains being replaced annually. The extent of serotype changes associated with these electropherotype changes is not known.

Neutralization tests with hyperimmune sera have identified at least six human rotavirus serotypes, and four of these have been shown to have worldwide distribution (3, 35). This classification has since been shown to have been based on simultaneous neutralization by antibodies to the two outer capsid viral proteins, VP4 (formerly VP3) and VP7 (22). This initial classification of serotype has been shown to correlate well with serological identification of VP7 (21).

The development of validated neutralizing monoclonal antibodies (MAbs) reacting specifically with the VP7 proteins of each of the four major human serotypes (1 to 4) has now permitted rapid direct typing of rotaviruses excreted in stools (13, 19, 30, 33). An assay developed in our laboratory further distinguishes antigenic differences (monotypes or subtypes) within VP7 of serotype 1 (10).

The importance of each human serotype in causation of disease from year to year in the same or different geographical areas has yet to be established. Surveys published to date from Europe (2, 18), North and South America (16, 23, 26), Africa (17), and Australia and Asia (1, 4, 7, 8, 25, 27, 31, 34) show fluctuations in the occurrence of serotypes 1, 2, 3, and 4 from country to country and temporally within each country. No consistent epidemiological patterns have been identified, possibly because most studies have been of limited duration (1 to 4 years). Temporal changes in predominant serotypes may explain the inconsistent results obtained in some of the recent trials of candidate rotavirus vaccines (14).

We present here the results from serotyping fecal rotavirus strains collected from children admitted to the gastroenteritis ward of the Royal Children's Hospital (RCH), Melbourne, Australia, from 1973 to 1989, and from newborn babies born and housed in Melbourne's obstetric hospital nurseries from 1974 to 1984. The results allow preliminary conclusions about periodicity of occurrence of some strains and assess the possible influence of neonatal strains on the epidemiology of strains causing severe disease in the same community. Information from other similar long-term surveys throughout the world is critically important for the development of an effective vaccine strategy.

## MATERIALS AND METHODS

All fecal specimens used in this survey had been initially collected from children within 48 h of admission to the hospital with acute diarrhea or from newborn babies aged 2 to 10 days. Rotavirus infection had been diagnosed by electron microscopy of negatively stained, concentrated stool homogenates or by enzyme immunoassay (25). Rotavirus-positive specimens had been stored at −70°C as unextracted feces, as 10% stool homogenates in phosphate-buffered saline, or as ultracentrifuge pellets resuspended in Tris buffer (32).

The first group of rotavirus-positive fecal specimens was

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TABLE 1. Occurrence of human rotavirus serotypes 1 to 4 in feces from children with acute diarrhea admitted to the hospital in Melbourne, Australia

| Yr | No. of specimens | | % Serotype[a]: | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rotavirus positive | Tested for serotype | 1a | 1c | 2 | 3 | 4 | Nontypeable |
| 1973 | 26[b] | 13 | 0 | 7.7 | 38.5 | 7.7 | 0 | 46.2 |
| 1974 | 181 | 72 | 1.4 | 43.1 | 0 | 11.1 | 16.7 | 26.4 |
| 1977–1979 | NK[c] | 32 | 0 | 6.3 | 62.5 | 6.3 | 3.1 | 15.6 |
| 1980 | 99 | 85 | 1.2 | 62.4 | 1.2 | 0 | 0 | 34.1 |
| 1981 | 82 | 51 | 7.8 | 31.4 | 2.0 | 0 | 13.7 | 45.1 |
| 1982 | 87 | 63 | 6.3 | 46.0 | 0 | 0 | 6.3 | 34.9 |
| 1983 | 60 | 54 | 16.7 | 44.4 | 3.7 | 3.7 | 0 | 18.5 |
| 1984 | 169 | 146 | 9.6 | 39.7 | 0 | 1.4 | 23.3 | 21.2 |
| 1985 | 130 | 102 | 66.7 | 2.9 | 1.0 | 1.0 | 5.9 | 22.5 |
| 1986 | 142 | 119 | 78.2 | 0 | 0 | 0.8 | 0 | 21.0 |
| 1987 | 98 | 36 | 86.1 | 0 | 0 | 0 | 0 | 11.1 |
| 1988 | 82 | 61 | 29.5 | 0 | 1.6 | 0 | 36.1 | 31.1 |
| 1989 | 133 | 109 | 16.5 | 0.9 | 14.7 | 2.8 | 18.3 | 33.9 |

[a] Serotypes 1b and 1d have been omitted.
[b] Specimens were collected from June to December.
[c] NK, Not known.

obtained from 943 children aged 9 days to 10 years admitted to the infectious disease ward of the RCH for treatment of severe acute diarrhea from June 1973 to December 1989. The RCH provides annual inpatient care for approximately 25,000 children residing in the Melbourne urban area. Although treatment of acute diarrhea has altered during that period to include the use of oral rehydration after admission to the hospital, there have been no major changes in the community-based treatment regimens or in criteria for admission to the hospital. The number of live births in urban Melbourne has remained stable at approximately 40,000 annually since 1976. The total numbers of children admitted annually to RCH with acute diarrhea in consecutive years from 1973 to 1989 included 540, 738, 606, 393, 424, 481, 468, 547, 573, 402, 326, 460, 356, 377, 345, 382, and 358, respectively. Annual numbers of rotavirus-positive specimens identified together with the number tested for serotype are shown in Table 1. The age ranges of patients included <6 months (77 children), 6 to 11 months (204 children), 12 to 17 months (233 children), 18 to 23 months (174 children), 24 to 35 months (156 children), 36 to 59 months (75 children), and >5 years (24 children). There was incomplete collection of specimens during 1973 and from 1977 to 1979. No specimens were available for serotyping from children admitted to the hospital in 1975 and 1976.

Rotavirus-positive fecal specimens were also obtained from 145 newborn babies (both symptomatic and asymptomatic) aged 2 to 10 days, housed in 2 nurseries providing special care and 13 nurseries providing routine antenatal care in 10 obstetric hospitals in Melbourne between 1974 and 1986. With the exception of one special care nursery, all babies examined had been delivered in the labor ward of the hospital in which they were housed. The majority of specimens (111 of 145) were obtained from babies born at the Royal Women's Hospital, Melbourne (RWH), during 1974 ($n$ = 1), 1975 ($n$ = 43), 1976 ($n$ = 5), 1977 ($n$ = 12), 1978 ($n$ = 7), 1979 ($n$ = 4), 1980 ($n$ = 18), 1981 ($n$ = 4), 1982 ($n$ = 7), 1983 ($n$ = 6), and 1984 ($n$ = 4). Sixty-eight of the 111 babies had been housed in the Special Care nursery of the RWH. The remaining 43 babies were housed in five nurseries for healthy full-term infants.

**Serotyping assay.** Stored specimens were thawed and used in a double-sandwich serotyping enzyme immunoassay by using hyperimmune rabbit antisera to human rotavirus as capture antisera and neutralizing MAbs specific for VP7 of the four major human rotavirus serotypes (1 to 4) as detector antibodies (13). The assay matches hyperimmune capture antisera raised to standard strains of serotypes 1 to 4 (RV4, RV5, RV3, and ST3) with detector antibodies reacting with VP7 of the same serotype. The assay has been validated by other techniques (19). Specimens that were initially nontypeable were concentrated approximately 2.5-fold with polyacrylamide gel (Lyphogel; Gelman Sciences, Inc.) and reassayed, at which point an additional 6% of specimens proved to be typeable (32).

Six serotype-specific MAbs that recognize VP7 of group A rotaviruses were used in the assay. MAbs RV4:1, RV4:2, and RV4:3 recognize different epitopes on VP7 of serotype 1 and permit intratypic classification into monotypes 1a, 1b, 1c, and 1d (10). MAbs RV5:3, RV3:1, and ST3:1 are specific for VP7 of serotypes 2, 3, and 4, respectively. MAb RV-A recognizes an inner capsid epitope on VP6 that is common to all group A rotaviruses and was incorporated as a control to estimate the amount of rotavirus antigen in each sample.

**Electrophoresis of rotavirus dsRNA.** Extracts of genomic double-stranded RNAs (dsRNAs) were prepared from 823 rotavirus-positive fecal specimens obtained from children admitted to the hospital between 1973 and 1988 and from 131 rotavirus-positive fecal specimens from newborn babies. dsRNAs from specimens obtained between 1973 and 1979 were prepared by the technique of Rodger et al. (29). Between 1980 and 1989, viral dsRNAs were prepared by phenol-chloroform-cresol extraction and stained with silver (10).

**Statistical analysis.** Results were analyzed by using the chi-square test, incorporating Yates' correction when appropriate.

## RESULTS

**Rotavirus serotypes identified in children admitted to the hospital with acute diarrhea.** It was possible to assign a serotype to 690 of 943 specimens tested (73.2%). The percentages of specimens assigned annually to serotype 1 (monotype 1a and 1c), 2, 3, or 4, and the percentages that were nontypeable are shown in Table 1. During the 15 years when specimens were available, serotype 1 strains were identified in 500 of 690 serotypeable strains (72.5%), comprising monotype 1a in 261 specimens (37.8%), monotype 1b in 18 (2.6%), monotype 1c in 218 (31.6%), and monotype 1d in 3 (0.4%). In the remaining specimens, serotype 2 was identified in 47 (6.8%), serotype 3 was identified in 20 (2.9%), and serotype 4 was identified in 106 (15.4%).

Rotavirus strains reacting with more than one serotype-specific MAb were identified in 22 of 690 specimens (3.2%). Five of these were serotype 3 strains that shared an epitope with VP7 of serotype 1 (12) and have been designated serotype 3 × 1 (see below). Eight specimens reacted as serotypes 1c and 4, two specimens reacted as serotypes 1b and 4, one specimen reacted as serotypes 1b and 2, two specimens reacted as serotypes 3 and 4, two specimens reacted as serotypes 1c and 2, one specimen reacted as serotypes 2 and 4, and one specimen reacted with all the MAbs used.

**Epidemiology of serotypes.** The monthly occurrence of

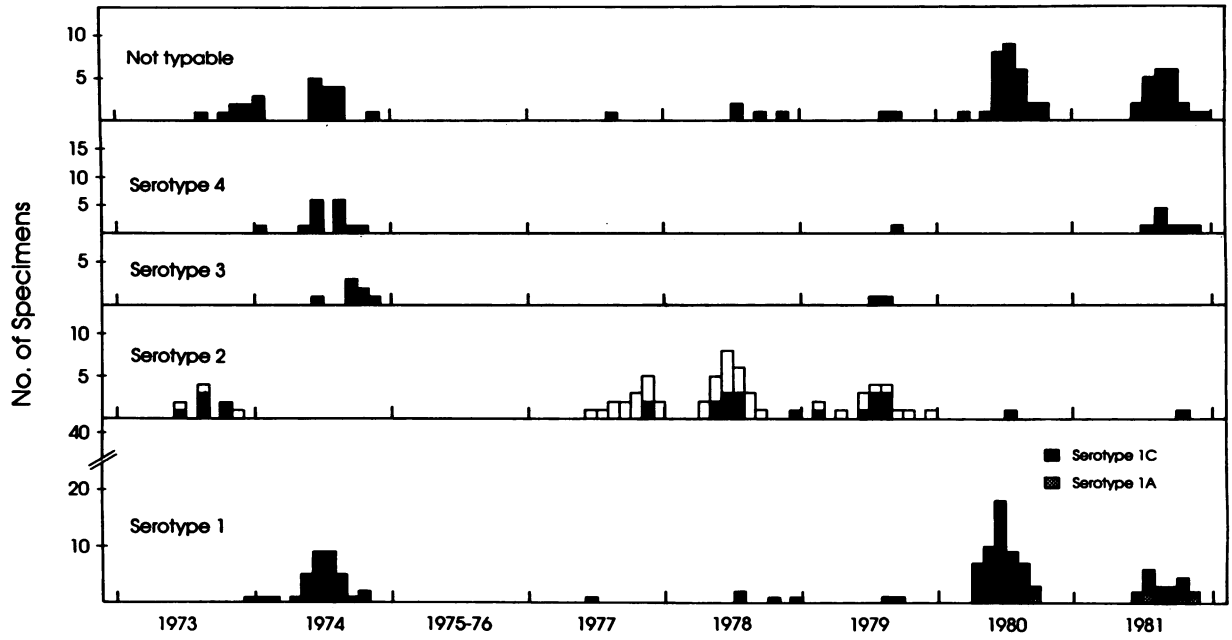


FIG. 1. Monthly occurrence of rotavirus serotypes and nontypeable strains excreted by Australian children with acute diarrhea admitted to one hospital from 1973 to 1981. Unshaded areas in serotype 2 represent strains with short-pattern electropherotypes (3).

each serotype during the winter epidemic seasons from 1973 to 1989 is shown in Fig. 1 and 2. No specimens from 1976 were available for study. Results for 1973 and for 1977 to 1979 are supplemented by serotype results predicted from electropherotype results available from an earlier survey that used some of the same specimens (29). Strains from this earlier survey had been extensively coelectrophoresed. Representative specimens of short-pattern strains were identified as serotype 2 by our serotyping system. We recorded serotype results deduced from electropherotypes (Fig. 1). Studies have shown that such extrapolation can be accurate with identical electropherotypes obtained within a particular geographical area (10, 25).

Of the 16 winter epidemics, 10 were associated with a clearly predominant serotype or monotype including serotype 2 (1973, 1977, 1978, and 1979), monotype 1a (1985,

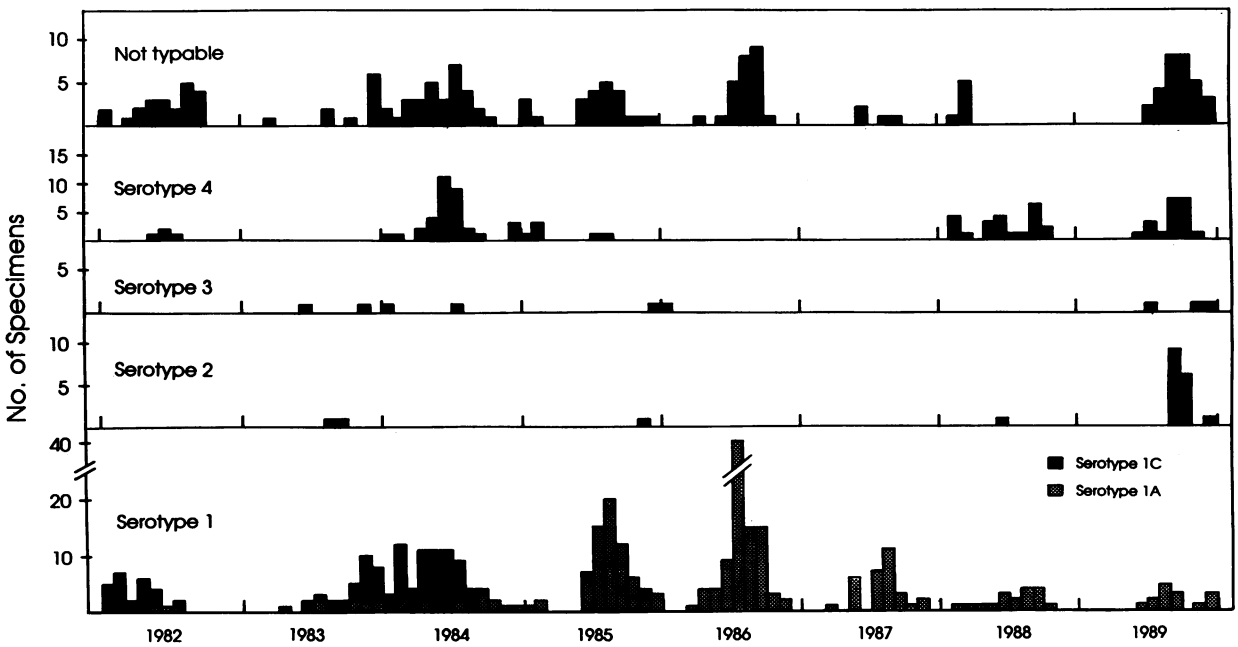


FIG. 2. Monthly occurrence of rotavirus serotypes and nontypeable strains excreted by Australian children with acute diarrhea admitted to one hospital from 1982 to 1989.

TABLE 2. Age range of children admitted to the hospital with diarrhea due to differing rotavirus serotypes

| Serotype | No. of children | % Infected in the following age group (mo): | | |
|---|---|---|---|---|
| | | <6 | 6–23 | >24 |
| 1a | 261 | 7.2 | 63.8 | 29.9 |
| 1c | 218 | 9.7 | 66.8 | 23.5 |
| 2 | 47 | 4.3 | 63.1 | 32.6 |
| 3 | 20 | 36.8 | 36.8 | 26.4 |
| 4 | 106 | 11.3 | 62.3 | 26.4 |

1986, and 1987), and monotype 1c (1980, 1982, and 1983) (Fig. 1 and 2; Table 1). Two serotypes coexisted during the winter epidemics of 1981 (1c and 4), 1984 (1c and 4), and 1988 (1a and 4). In 1974, three serotypes (1c, 3, and 4) were common in the community, and all four serotypes (1a, 1b, 2, 3, and 4) were identified during 1989. Serotypes 1c, 2, 3, and 4 probably coexisted during 1975, since serotyped strains with identical electropherotypes were found in 1974.

The monthly occurrence of nontypeable strains (Fig. 1 and 2) reflected the monthly patterns of admissions for acute diarrhea.

The mean and median numbers of children admitted to the hospital with rotavirus diarrhea during 1981, 1984, 1988, and 1989, when more than one serotype was common (119 and 108, respectively), were similar to the mean and median numbers admitted during 1980, 1982, 1983, 1985, 1986, and 1987, when one clearly predominant serotype was identified (103 [mean] and 99 [median] children). The mean and median numbers of children admitted with rotavirus diarrhea during 1981, 1982, 1984, 1985, 1988, and 1989, when serotypes 1 and 4 coexisted (105 [mean] and 104 [median]), did not differ from mean and median numbers admitted during 1980, 1983, 1986, and 1987 (100 [mean] and 99 [median]), when serotype 4 strains were absent.

Serotype 1 strains were dominant during at least 11 of the 16 winter periods for which data were available. Monotype 1c strains were common during 1974 and 1975, were occasionally identified during 1977 to 1979, and replaced serotype 2 strains in 1980. Monotype 1c strains present in the community from 1980 to 1984 were replaced by monotype 1a strains after 1984.

Serotype 2 strains were common during the winter of 1973, were overwhelmingly predominant during the three successive winters of 1977 to 1979, and were then rarely identified until the winter of 1989. With the exceptions of the winters of 1974 and 1989, serotype 3 strains have been rarely identified as a cause of severe diarrhea in children in Melbourne.

Serotype 4 strains appeared intermittently throughout the 16 years studied including 1974, 1981, 1982, 1984, 1985, 1988, and 1989. Since 1980, serotype 4 strains have recurred as a cause of severe diarrhea every 2 to 3 years. The initial appearance has been followed by a decrease in occurrence during the following year.

**Age distribution in relation to rotavirus serotype.** The age ranges of children admitted to the hospital with diarrhea due to each serotype are shown in Table 2. Within each serotype, the majority of children infected were aged 6 to 23 months with the exception of serotype 3, which was found as frequently in infants less than 6 months old as in children aged 6 to 23 months.

There was no evidence that the appearance of a new serotype during a winter epidemic was associated with an increase in the numbers of children of older age groups admitted to the hospital. From 1980 to 1989, 1,082 children were admitted to the hospital with rotavirus diarrhea, of which 284 were more than 2 years old. The proportion (181 of 735) of children aged more than 2 years admitted during winter epidemics when a new serotype appeared in the community (1980, 1981, 1984, 1985, 1988, and 1989) did not differ significantly from the proportion (103 of 347) admitted during the years 1982, 1983, 1986, and 1987, when no change in serotype was observed ($\chi^2 = 3.25$, $P > 0.05$).

**Rotavirus electropherotypes.** An electropherotype was identified in 416 of 735 specimens available for study. All serotypes exhibited genomic RNA polymorphism, including serotype 2 (11 electropherotypes), serotype 1a (20 electropherotypes), serotype 1c (13 electropherotypes), serotype 1b (2 electropherotypes), serotype 3 (8 electropherotypes), and serotype 4 (8 electropherotypes). The predominant electropherotype patterns of serotypes 1a and 1c changed annually, although some electropherotypes persisted for more than 1 year. The strain of serotype 1a that replaced serotype 1c as the dominant strain in 1985 was first detected in 1984. Coelectrophoresis of these two strains indicated differences in mobilities in 10 of the 11 gene segments (10). The electropherotypes of serotype 4 strains found during each outbreak differed from each other and comprised more than one electropherotype in at least two of the five outbreaks. An electropherotype was determined for five serotype 3 strains (including two from children aged 9 and 12 days) and two serotype 3 × 1 strains. All showed RNA patterns that differed from each other and from the serotype 3 or 3 × 1 strains endemic in obstetric hospital nurseries (see below).

Gel electrophoresis of genomic RNAs of 88 of 253 nontypeable specimens obtained in 1973 ($n = 3$), 1974 ($n = 8$), 1980 ($n = 4$), 1981 ($n = 4$), 1982 ($n = 3$), 1983 ($n = 10$), 1984 ($n = 26$), 1985 ($n = 4$), 1986 ($n = 10$), 1987 ($n = 10$), and 1988 ($n = 6$) revealed either no RNA (50) or patterns identical with typeable strains present concurrently in the community (38).

There were sufficient amounts of specimens remaining to perform gel electrophoresis on 7 of the 17 strains reacting with more than one MAb (excluding serotype 3 × 1 strains). The electropherotype pattern revealed more than 11 bands in all seven specimens, indicating mixed infections.

**Rotavirus serotypes in newborn babies.** Serotypes could be assigned to 135 of 145 specimens from newborn babies (93%). All belonged to serotype 3. Some strains that reacted positively with the serotype 3-specific antibody (MAb RV3:1) were further classified as also reacting positively (as judged by optical density readings) with a serotype 1 MAb (RV-4:3), but at 10- to 100-fold-lower optical density readings than those obtained with RV4:3 by homotypic serotype 1 viruses (12). These serotype 3 neonatal strains possess an epitope shared with VP7 of serotype 1 and are designated serotype 3 × 1. Overall, 44 of 103 rotaviruses identified from babies at RWH (42.7%) and 17 of 32 strains from the other hospitals (53.1%) were serotype 3 × 1.

From 1974 to 1977, serotype 3 × 1 comprised 52 of 82 strains (63.4%) but comprised only 9 of the 53 strains identified from 1978 to 1984 (17.0%) ($\chi^2 = 30.1$, $P < 0.001$).

## DISCUSSION

The results of this survey document the epidemiology of rotavirus serotypes in causation of severe disease in one large urban area since the original detection of this virus in

1973. Serotype 1 strains are confirmed as the single most important cause of rotavirus disease throughout the world. The epidemiological importance of subdivision of serotype 1 strains into monotypes 1a and 1c is emphasized by the results of this study. Monotype 1a strains replaced 1c strains after 1984 and persisted for the duration of the survey. Monotype 1a strains possess a neutralization epitope not present in 1c strains (10). That may have favored their emergence in the community after the dominance of monotype 1c strains from 1980 to 1984. These monotypes may not only be of local importance, since strains isolated in the United States (Wa) and in Japan (KU and K8), have been identified as monotype 1a and monotype 1c, respectively (10).

During the last 10 years of the survey, serotype 4 strains showed an episodic pattern of appearance, characteristically peaking in children admitted to the hospital during one winter, persisting the following winter in reduced numbers, and then disappearing from the community for one to two winters, when they reemerged with changed electropherotypes. Epidemic occurrences of serotype 4 have been noted in the United Kingdom (2), Thailand (27), Mexico (26), and Japan (34), but no periodicity has been reported to date.

No predictable patterns of occurrence were seen with serotypes 2 or 3. The period studied was marked by the emergence of a serotype 2 strain in 1977 as the dominant serotype and its persistence with a constant electropherotype for 3 years. The dominance of this strain is apparent from serotyping results, reinforced by extrapolation of serotype from electropherotype results. The latter imposes some limitations on interpretation of the data. Nevertheless, such extrapolation has been shown to be accurate with identical electropherotypes obtained in a limited geographical area (10, 25). Serotype 2 strains with changed electropherotypes reemerged as a cause of severe diarrhea in children 10 years later. Intermittent epidemics of strains with short-pattern electropherotypes (presumably serotype 2) have been noted previously in many communities in developed and developing countries (15). Serotype 3 strains were rarely identified in children admitted to the hospital in Melbourne after 1974 and have been uncommon in many other surveys.

Throughout the years of this survey, the percentages of nontypeable strains varied annually from 11 to 46%. Their monthly occurrence reflected the monthly patterns of serotypes concurrently identified among hospital admissions. Overall, the assay failed to identify the VP7 type of 27% of rotaviruses. It is likely that these specimens contained insufficient viral antigen (VP7) available for recognition by the MAbs. The ability to determine serotype with this assay has been shown to be influenced by the number and condition of viruses in the specimen (32). When it was possible to determine the electropherotype of a nontypeable specimen, the pattern was always identical with that of typeable strains present concurrently in the community. It is also possible that untyped specimens represented new, as yet unidentified, rotavirus serotypes. Incorporation of mixtures of MAbs to extend the range of VP7 epitopes identified may improve the sensitivity of the assay. In addition, highly sensitive techniques involving amplification of genetic material by the polymerase chain reaction method may be required for full identification of rotaviruses present in feces.

There was little evidence that changes in serotype appeared as a "herald wave" during the end of the preceding winter, as has been described for influenza (20). The numbers of patients sampled may have been below the threshold of detection for this effect. The monotype 1a serotype that supplanted monotype 1c in 1985 had been present as an uncommon cause of disease in the community during the 4 years preceding its emergence as a dominant strain. The appearances of serotype 4 strains in 1981, 1984, and 1988 were not signalled during the preceding winters.

Approximately 60,000 children less than 2 years of age reside annually in the area served by RCH. Despite this large population of children and the genetic and serotypic diversity of strains causing severe infections, there was little evidence of severe sequential rotavirus infections. During the survey, only one child who required two hospital admissions (in successive winters) was identified. Winter epidemics involving two or more serotypes did not result in increased numbers of children admitted with severe diarrhea (or in increased numbers of older children admitted) compared with winters when a single serotype was dominant. These observations imply that some measure of clinical cross-protection exists between serotypes. Longitudinal surveillance studies of rotavirus diarrhea in children after primary rotavirus infection (of neonates or older children) support the possibility of heterologous clinical protection against severe symptoms on reinfection (6, 28). The increased resistance to disease observed in children may be due to physiological maturity of the gut, although severe disease has been recorded in adults (5) and we have observed severe rotavirus infection in 31 of 206 children more than 5 years of age admitted to the hospital for treatment of diarrhea (unpublished observation).

By comparison with the diversity observed in strains excreted by hospitalized children, all rotavirus strains endemic in neonatal nurseries of at least one large Melbourne obstetric hospital between 1974 and 1984 belonged to serotype 3. Whether serotype 3 rotaviruses were present in neonatal nurseries prior to 1974 is not known. It is possible that the existence of this strain in newborn nurseries influenced the occurrence of serotype 3 strains as a cause of severe diarrhea in the Melbourne community at large, since serotype 3 infection rarely occurred in Melbourne after 1975. Children infected with serotype 3 as newborn babies have been shown to be clinically immune to severe disease on reinfection (6). The reemergence of serotype 3 strains in the community in 1989 may have been epidemiologically associated with the observation that no rotavirus strains have been identified in newborn babies in the RWH nurseries since 1987 (16a).

It is also possible that the strain endemic in newborn nurseries had a profound effect on epidemiology of rotavirus diarrhea of other serotypes in the community at large. From 1974 to 1977, the majority of neonatal strains possessed an epitope on VP7 that was shared with VP7 of serotype 1. During these years, serotypes 1 and 4 were rarely encountered in children admitted to the hospital with rotavirus diarrhea. Neonatal infection may have conferred clinical protection against serotype 1 via antibody to the shared epitope on VP7. Protection against serotype 4 disease may have been conferred by a further unidentified shared epitope or epitopes (9, 24). Alteration in VP7 epitopes of the neonatal strain dominant after 1977 (11), such that reactivity due to the shared serotype 1 epitope was lost, was followed 2 years later by the reappearance of serotype 1 and then serotype 4 as causes of severe diarrhea in children in the community.

The results of this survey provide only circumstantial evidence for the influence of neonatal infection on epidemiological patterns and for the existence of clinical cross-protection between rotavirus serotypes. The hypothesis is also entirely based on observations of change in the VP7

type. The potential role of VP4 changes in influencing such longitudinal studies cannot yet be evaluated. The survey does not permit conclusions about rotavirus serotypes that may serve to initiate or to boost immunity by causing mild diarrhea in the same community. Cumulative results of longitudinal studies (in developed and developing countries) and of current vaccine field trials with a human rotavirus strain (24) are required before the evidence for heterotypic cross-protection in humans can be evaluated. If heterotypic protection against clinically severe disease is induced after natural infection (in humans), then it may be possible to simplify current vaccine strategies.

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## REFERENCES

1. **Ahmed, M. U., K. Taniguchi, N. Kobayashi, T. Urasawa, F. Wakasugi, M. Islam, H. Shaikh, and S. Urasawa.** 1989. Characterization by enzyme-linked immunosorbent assay using subgroup- and serotype-specific monoclonal antibodies of human rotavirus obtained from diarrheic patients in Bangladesh. J. Clin. Microbiol. **27**:1678–1681.
2. **Beards, G. M., U. Desselberger, and T. H. Flewett.** 1989. Temporal and geographical distribution of human rotavirus serotypes, 1983 to 1988. J. Clin. Microbiol. **27**:2827–2833.
3. **Beards, G. M., J. N. Pilfold, M. E. Thouless, and T. H. Flewett.** 1980. Rotavirus serotypes by serum neutralisation. J. Med. Virol. **5**:231–237.
4. **Birch, C. J., R. L. Heath, and I. D. Gust.** 1988. Use of serotype-specific monoclonal antibodies to study the epidemiology of rotavirus infection. J. Med. Virol. **24**:45–53.
5. **Bishop, R. F.** 1986. Epidemiology of diarrhoeal disease caused by rotavirus, p. 158–170. *In* J. Holmgren, A. Lindberg, and R. Mollby (ed.), Development of vaccines and drugs against diarrhea. Studentlitteratur, Lund, Sweden.
6. **Bishop, R. F., G. L. Barnes, E. Cipriani, and J. S. Lund.** 1983. Clinical immunity after neonatal rotavirus infection. N. Engl. J. Med. **309**:72–76.
7. **Bishop, R. F., L. E. Unicomb, Y. Soenarto, H. Suwardji, Ristanto, and G. L. Barnes.** 1989. Rotavirus serotypes causing acute diarrhoea in hospitalised children in Yogyakarta, Indonesia during 1978–1979. Arch. Virol. **107**:207–214.
8. **Brown, D. W. G., M. M. Mathan, M. Mathew, R. Martin, G. M. Beards, and V. I. Mathan.** 1988. Rotavirus epidemiology in Vellore, South India: group, subgroup, serotype, and electropherotype. J. Clin. Microbiol. **26**:2410–2414.
9. **Chiba, S., T. Yokoyama, S. Nakata, Y. Morita, T. Urasawa, K. Taniguchi, S. Urasawa, and T. Nakao.** 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. Lancet **ii**:417–421.
10. **Coulson, B. S.** 1987. Variation in neutralization epitopes of human rotaviruses in relation to genomic RNA polymorphism. Virology **159**:209–216.
11. **Coulson, B. S., K. J. Fowler, R. F. Bishop, and R. G. H. Cotton.** 1985. Neutralizing monoclonal antibodies to human rotavirus and indications of antigenic drift among strains from neonates. J. Virol. **54**:14–20.
12. **Coulson, B. S., J. M. Tursi, W. J. McAdam, and R. F. Bishop.** 1986. Derivation of neutralizing monoclonal antibodies to human rotaviruses and evidence that an immunodominant neutralization site is shared between serotypes 1 and 3. Virology **154**:302–312.
13. **Coulson, B. S., L. E. Unicomb, G. A. Pitson, and R. F. Bishop.** 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. J. Clin. Microbiol. **25**:509–515.
14. **Edelman, R.** 1987. Perspective on the development and deployment of rotavirus vaccines. Pediatr. Infect. Dis. J. **6**:704–710.
15. **Estes, M. K., D. Y. Graham, and D. H. Dimitrov.** 1984. The molecular epidemiology of rotavirus gastroenteritis. Prog. Med. Virol. **29**:1–22.
16. **Flores, J., K. Taniguchi, K. Green, I. Perez-Schael, D. Garcia, J. Sears, S. Urasawa, and A. Z. Kapikian.** 1988. Relative frequencies of rotavirus serotypes 1, 2, 3, and 4 in Venezuelan infants with gastroenteritis. J. Clin. Microbiol. **26**:2092–2095.

16a. **Garland, S.** Personal communication.

17. **Georges-Courbot, M. C., A. M. Beraud, G. M. Beards, A. D. Campbell, J. P. Gonzalez, A. J. George, and T. H. Flewett.** 1988. Subgroups, serotypes, and electropherotypes of rotavirus isolated from children in Bangui, Central African Republic. J. Clin. Microbiol. **26**:668–671.
18. **Gerna, G., S. Arista, N. Passarani, A. Sarasini, and M. Battaglia.** 1987. Electropherotype heterogeneity within serotypes of human rotavirus strains circulating in Italy. Arch. Virol. **95**:129–135.
19. **Gerna, G., N. Passarani, L. E. Unicomb, M. Parea, A. Sarasini, M. Battaglia, and R. F. Bishop.** 1989. Solid-phase immune electron microscopy and enzyme-linked immunosorbent assay for typing of human rotavirus strains using monoclonal antibodies: a comparative study. J. Infect. Dis. **159**:335–339.
20. **Glezen, W. P., R. B. Couch, and H. R. Sise.** 1982. The influenza herald wave. Am. J. Epidemiol. **116**:589–598.
21. **Green, K. Y., J. F. Sears, K. Taniguchi, K. Midthun, Y. Hoshino, M. Gorziglia, K. Nishikawa, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1988. Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. J. Virol. **62**:1819–1823.
22. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities ($VP_3$ and $VP_7$) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**: 8701–8704.
23. **Linhares, A. C., Y. B. Gabbay, J. D. P. Mascarenhas, R. B. Freitas, T. H. Flewett, and G. M. Beards.** 1988. Epidemiology of rotavirus subgroups and serotypes in Belem, Brazil: a three year study. Ann. Inst. Pasteur Virol. **139**:89–99.
24. **Morita, Y., K. Taniguchi, T. Urasawa, and S. Urasawa.** 1988. Analysis of serotype-specific neutralization epitopes on $VP_7$ of human rotavirus by use of neutralizing monoclonal antibodies and antigenic variants. J. Gen. Virol. **69**:451–458.
25. **Nakagomi, T., K. Akatani, N. Ikegami, N. Katsushima, and O. Nakagomi.** 1988. Occurrence of changes in human rotavirus serotypes with concurrent changes in genomic RNA electropherotypes. J. Clin. Microbiol. **26**:2586–2592.
26. **Padella-Noriega, L., C. F. Arias, S. Lopez, F. Puerto, D. R. Snodgrass, K. Taniguchi, and H. B. Greenberg.** 1990. Diversity of rotavirus serotypes in Mexican infants with gastroenteritis. J. Clin. Microbiol. **18**:1114–1119.
27. **Pongsuwanne, Y., K. Taniguchi, M. Choonthanom, M. Chiwakul, T. Susansook, S. Saquannongse, C. Jayavasu, and S. Urasawa.** 1989. Subgroup and serotype distributions of human, bovine and porcine rotavirus in Thailand. J. Clin. Microbiol. **27**:1956–1960.
28. **Reves, R. R., M. M. Mohassin, K. Midthun, A. Z. Kapikian, T. Naguib, A. M. Zaki, and H. L. Du Pont.** 1989. An observational study of naturally acquired immunity to rotaviral diarrhea in a cohort of 363 Egyptian children. Calculation of risk for second episodes using age-specific person-years of observation. Am. J. Epidemiol. **130**:981–988.
29. **Rodger, S. M., R. F. Bishop, C. Birch, B. McLean, and I. H. Holmes.** 1981. Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979, as determined by electrophoresis of genome ribonucleic acid. J. Clin. Microbiol. **13**:272–278.
30. **Taniguchi, K., T. Urasawa, Y. Morita, S. Urasawa, and H. B. Greenberg.** 1987. Direct serotyping of human rotavirus in stools

by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3- and 4-specific monoclonal antibodies directed to $VP_7$. J. Infect. Dis. **155**:1159–1166.

31. **Unicomb, L. E., and R. F. Bishop.** 1989. Epidemiology of rotavirus strains infecting children throughout Australia during 1986–1987: a study of serotype and RNA electropherotype. Arch. Virol. **106**:23–34.
32. **Unicomb, L. E., B. S. Coulson, and R. F. Bishop.** 1989. Experience with an enzyme immunoassay for serotyping human rotaviruses. J. Clin. Microbiol. **27**:586–588.
33. **Urasawa, S., T. Urasawa, K. Taniguchi, Y. Morita, N. Sakurada, Y. Saeki, O. Morita, and S. Hasegawa.** 1988. Validity of an enzyme-linked immunosorbent assay with serotype-specific monoclonal antibodies for serotyping human rotavirus in stool specimens. Microbiol. Immunol. **32**:699–708.
34. **Urasawa, S., T. Urasawa, K. Taniguchi, F. Wakasugi, N. Kobayashi, S. Chiba, N. Sakurada, M. Morita, O. Morita, M. Tokieda, H. Kawamoto, Y. Minekawa, and M. Ohseto.** 1989. Survey of human rotavirus serotypes in different locales in Japan by enzyme-linked immunosorbent assay with monoclonal antibodies. J. Infect. Dis. **160**:44–51.
35. **Wyatt, R. G., H. D. James, Jr., A. L. Pittman, Y. Hoshino, H. B. Greenberg, A. R. Kalica, J. Flores, and A. Z. Kapikian.** 1983. Direct isolation in cell culture of human rotaviruses and their characterization into four serotypes. J. Clin. Microbiol. **18**:310–317.