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# Molecular Epidemiology of Rotaviruses Associated with Pediatric Diarrhea in Bangkok, Thailand

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**Rotavirus diarrhea in 453 pediatric patients (29.8% of 1,518) was studied in greater Bangkok during 1985 to 1987. The disease persisted all year, increasing in incidence from August to January (30 to 50%). Polyacrylamide gel electrophoresis of rotavirus RNA from these patients and from an additional 46 patients of a 1982 to 1983 epidemic revealed 26 electropherotypes, 4 with short (S) and 22 with long (L) RNA profiles. Of the analyzed specimens, 85.5% were L forms. Only one or a few electropherotypes predominated in each epidemic, whereas others appeared sporadically at low frequencies. Shifts in the predominant electropherotypes were observed in every epidemic. Of these, 126 strains were tested for subgroup and serotype by monoclonal antibody enzyme immunoassay. Serotype 4 prevailed from 1982 to 1983, while serotype 1 was encountered more frequently than serotypes 2 and 4 from 1985 to 1987. A complete correlation was found between the electrophoretic migration of segments 10 and 11 and the serologically defined subgroup specificity. Distinct electropherotypes occurred within the same serotype, and strains with the identical electropherotype always showed the same serotype specificity. No specific electropherotype or serotype correlated with patient age. In this study, atypical rotaviruses and mixed infections with different rotaviruses were identified.**

Rotavirus gastroenteritis is a worldwide disease affecting primarily infants, young children, and the young of a wide variety of mammalian and avian species (9). The most frequently isolated rotaviruses share common group A antigens. It has definitely been shown that group A human rotaviruses can be classified into two subgroups (7, 38) and at least four distinct serotypes (37). This antigenic complexity and the difficulty of cultivating rotaviruses isolated from humans has hampered serologic characterization of human virus strains and seroepidemiologic surveys of rotavirus outbreaks. Molecular techniques, such as analysis of the electrophoretic mobility of the 11 double-stranded RNA segments of rotaviruses by polyacrylamide gel electrophoresis (PAGE), are now most commonly used for epidemiological studies (8). PAGE permits a preliminary differentiation of the two subgroups of human rotaviruses on the basis of the different electrophoretic mobilities of segments 10 and 11 (7). It has been subsequently been widely employed to determine the electropherotypes of human rotavirus strains circulating in different geographical areas, often providing important epidemiological information (21, 25, 30, 34). Such studies have revealed an extensive genomic diversity; for example, 19 electropherotypes were distinguished in Australia (25) and 29 electropherotypes were found in France (21).

The aim of the present study was to investigate the occurrence and circulation of different human rotavirus electropherotypes from diarrheic patients who visited three hospitals in greater Bangkok during 1982 to 1983 and 1985 to 1987. This was the first study of the molecular epidemiology of human rotaviruses in Thailand, where rotavirus disease is typically endemic. The association between subgroup and serotype specificity and RNA electropherotype was also examined.

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## MATERIALS AND METHODS

**Clinical specimens.** Fecal samples (July 1985 to June 1986) were collected from 951 children under 15 years of age with clinical diagnosis of acute diarrhea admitted to Siriraj Hospital and Children's Hospital in central Bangkok and to Bamrasnaradura Infectious Diseases Hospital in Nonthaburi district. Samples from July 1986 to July 1987 were from 567 patients under 24 months of age admitted to the Children's Hospital. An additional 46 rotavirus strains were collected during a 1982 to 1983 epidemic (35). All specimens were stored at −20°C before being examined for rotavirus infection.

The prototype rotavirus Wa (serotype 1) and S2 (serotype 2) strains were obtained from A. R. Bellamy, Department of Cell Biology, University of Aukland, Auckland, New Zealand.

**Rotavirus diagnosis.** Rotaviruses in stool filtrates were determined by enzyme-linked immunosorbent assay (ELISA) (153 specimens) or by PAGE (61 specimens) or by both (1,304 specimens). The techniques used have been described elsewhere (16).

**RNA analysis.** RNA genome analysis was performed on 234 (75.5%), 113 (79%), and 46 (39.7%) rotavirus-positive samples detected during the 1985 to 1986, 1986 to 1987, and 1982 to 1983 epidemics, respectively. PAGE was based on the techniques of Herring et al. (12) and Rodger and Holmes (26). Double-stranded genome RNA of rotavirus present in stools or in tissue culture fluids was extracted with phenol-chloroform-isoamyl alcohol. Fecal material (0.25 g) was suspended in 0.5 ml of 0.1 M sodium acetate buffer (pH 5.0) containing 1% (wt/vol) sodium dodecyl sulfate, and an equal volume of a 3:2 (vol/vol) phenol-chloroform mixture was added. The mixture was shaken vigorously in a 1.5-ml microcentrifuge tube for 1 min on a Vortex mixer and centrifuged in a microcentrifuge at 6,000 to 7,000 rpm for 2 min. The clear upper aqueous layer containing double-stranded RNA was removed and a 40-μl aliquot was then

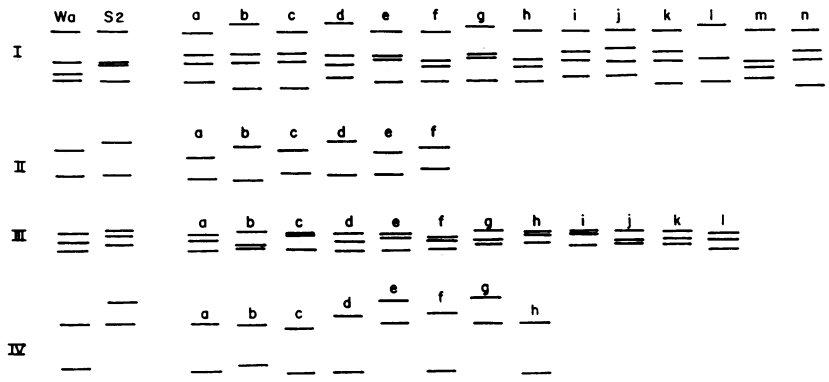


FIG. 1. Schematic patterns of migration of the 11 segments of double-stranded RNA from rotavirus strains detected in diarrheic patients in Bangkok during the periods 1982 to 1983 and 1985 to 1987. This classification scheme was based on that proposed by Lourenco et al. (21) for use in comparing rotavirus electropherotypes. All of the patterns of migration of RNA segment groups I to IV are sketched. The left two lanes represent the electropherotypes of the reference rotavirus strains Wa and S2. Differences in the relative migration of RNA bands within a group are indicated by lowercase letters.

mixed with 15 μl of sample buffer (0.5 M Tris [pH 6.8], 25% glycerol, 0.2% bromphenol blue) before being applied to gel for electrophoresis.

Electrophoresis was done in slab polyacrylamide gel. A Laemmli discontinuous system (18) with sodium dodecyl sulfate omitted from all the buffers and a 10% polyacrylamide separating gel with a 3% stacking gel was used. The gel plate was 25 cm long by 18 cm wide with 15 wells spaced 0.75 mm apart. Fifty microliters of each RNA preparation was carefully loaded into each well. Electrophoresis was carried out at room temperature for 18 h at a constant current of 18 mA per slab gel. The separated double-stranded RNAs in the slab gel were visualized by silver staining as previously described (12).

Variation in RNA migration was demonstrated by parallel comparison of the RNA patterns on the same gel and by coelectrophoresis which included those strains yielding identical patterns.

**Classification of rotavirus electropherotypes.** To facilitate systematic comparison of the large number of viral RNA patterns encountered, the following classification method was used. First, at the diagnosis step, the long pattern with the faster migration of segments 10 and 11 was designated L as opposed to the short pattern, which was referred to as the S electropherotype (7, 15). When all the RNA segments were resolved, the electropherotypes were classified by dividing the 11 RNA segments into four groups by the method of Lourenco et al. (21) and electropherotypes were identified as combinations of the variations within each group (Fig. 1). Differences in the relative migration of RNA bands within a group were indicated by small letters. The electropherotype of an isolate was expressed as the combination of its patterns (Table 1). For practical reasons, each electropherotype was given a designation consisting of the year and month of its first sample collection followed by L or S, referring to the L or S electropherotype, respectively. The number after L or S showed the strain number detected that year (Table 1).

**Subgrouping and serotyping tests.** A double-antibody sandwich ELISA was used (14, 23). The tests were performed at the Clinical Research Institute, Osaka National Hospital, Osaka, Japan. The monoclonal antibodies employed were Osaka National Hospital AH6 for capture antibody, AB4 for detective antibody, AB22 for subgroup I, AC10 for subgroup II, AH49 for serotype 1, AG12 for serotype 2, AC5 for serotype 3, and AE18 for serotype 4. Virus suspension was inoculated onto MA104 cells if virus isolation was required, and the cell culture supernatant was used in the ELISA.

## RESULTS

**Rotavirus infections among diarrheic patients from 1985 to 1987.** From a total of 951 assayed stool specimens of diarrheic cases from July 1985 to June 1986, 310 specimens (32.6%) were rotavirus positive. From 567 cases during the July 1986 to July 1987 period, 143 specimens (25.2%) were positive. The monthly distribution of rotavirus-positive diarrheas is shown in Fig. 2. They were detected throughout the year, with the incidence increasing in August and reaching a peak (30 to 50%) in December and January. In February, the infection rate dropped abruptly and remained at a low level (7 to 26%) through the nonepidemic period (February to July), which covered the entire hot season. Such a seasonal pattern of pediatric rotavirus diarrhea occurred during the 1982 to 1983 epidemic (35).

Study during the 1985 to 1986 period revealed a high rate of rotavirus infection (23 to 40%) among patients under 4 years of age (Table 2). However, most of the diarrheic patients (920 of 941, 97.8%) were under 2 years; hence, our subsequent study from 1986 to 1987 was limited to subjects under 2 years of age. The distribution of rotavirus infections (1985 to 1987) among children of different ages up to 2 years is shown in Table 3 in which it can be seen that most are under 12 months old; a significant reduction of diarrheic cases was seen in older children. The rotavirus infection rate was relatively low among infants under 3 months old, but rose to a peak in the 7- to 9-month age group. The pattern was similar when individual rotavirus epidemics were analyzed separately (data not shown).

**Diversity of electropherotypes.** Migration patterns in the RNA segment groups I, II, III, and IV fell into 14, 6, 12, and 8 patterns, respectively (Fig. 1). The rotavirus strains could be differentiated into 26 different electropherotypes, 22 with L and 4 with S electropherotypes. The types and relative frequencies of occurrence are shown in Table 1.

**Epidemic distribution of electropherotypes.** The yearly and monthly distributions of electropherotypes are summarized in Table 1 and Fig. 3, respectively. Nine electropherotypes were identified during the July 1982 to March 1983 period. Electropherotype 82/9-L2 predominated (32 of 46, 69.6%), whereas the other eight electropherotypes appeared sporad-

TABLE 1. Classification and yearly distribution of 26 different RNA electropherotypes of human rotaviruses detected in stools obtained from diarrheic patients in greater Bangkok during 1982 to 1983 and 1985 to 1987

| Electropherotype designation | RNA migration pattern in indicated segment group[a] | | | | Serotype[b] | No. of samples classified | | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | | 1982–1983 | 1985–1986 | 1986–1987 | Total |
| 82/8-L1 | c | c | f | a | NT[c] | 3 | | | 3 |
| 82/9-L2 | c | c | f | d | 4 | 32 | | | 32 |
| 82/10-L3 | n | c | e | a | 1 | 2 | | | 2 |
| 82/11-L4 | b | c | f | d | NT | 1 | | | 1 |
| 82/11-L5 | c | c | f | f | 4 | 2 | | | 2 |
| 82/12-L6 | a | a | a | a | 1 | 1 | 93 | 4 | 98 |
| 83/1-L1 | k | e | a | a | NT | 1 | | | 1 |
| 83/1-L2 | c | c | f | c | NT | 1 | | | 1 |
| 85/7-L1 | a | a | e | a | 1 | | 65 | 74 | 139 |
| 85/7-L2 | d | f | c | d | 4 | | 8 | 8 | 16 |
| 85/7-L3 | a | a | c | a | 1 | | 15 | | 15 |
| 85/8-L4 | l | b | b | c | 4 | | 1 | | 1 |
| 85/8-L5 | a | a | d | a | NT | | 5 | 4 | 9 |
| 85/9-L6 | i | a | e | c | NT | | 1 | | 1 |
| 85/9-L7 | i | a | e | a | NT | | 1 | | 1 |
| 85/12-L8 | i | a | a | a | 1 | | 1 | | 1 |
| 86/4-L1 | e | a | a | a | 1 | | 1 | | 1 |
| 86/4-L2 | g | b | k | h | NT | | 1 | | 1 |
| 86/6-L3 | a | f | e | c | 1 | | 1 | 2 | 3 |
| 86/6-L4 | m | f | c | b | 4 | | 2 | 3 | 5 |
| 86/12-L5 | a | a | e | c | NT | | | 2 | 2 |
| 87/2-L1 | j | f | l | h | NT | | | 1 | 1 |
| 82/7-S1 | f | d | i | g | NT | 3 | | | 3 |
| 85/7-S1 | h | d | h | e | 2 | | 37 | | 37 |
| 85/11-S2 | h | d | j | e | 2 | | 2 | | 2 |
| 86/10-S1 | f | d | g | e | 2 | | | 15 | 15 |
| Total | | | | | | 46 | 234 | 113 | 393 |

[a] See Fig. 1.
[b] Only representative sample(s) of each electropherotype were analyzed for serotype as shown in Table 4.
[c] NT, Not tested.

ically with very low frequency. Of these, only 82/12-L6 reappeared in 1985 to 1986 when it was the predominant type and persisted, tapering off in the 1986 to 1987 season. During the July 1985 to June 1986 period, 15 electropherotypes were characterized. Again, most (11 electropherotypes) were detected sporadically. Types 85/7-L1 and 85/7-S1 shared dominance with 82/12-L6, although at a lower frequency. Electropherotype 85/7-L3 was detected during the rainy season (Fig. 3, left). The 85/7-L1, 85/7-S1, and 85/7-L3 electropherotypes were detected throughout the 1985 to 1986 epidemic, but the last two types suddenly disappeared in 1986 to 1987. In contrast, 85/7-L1 continued through the nonepidemic months and became the only major cause of epidemic in 1986 to 1987 (74 of 113 cases, 65.5%), during which the remaining eight electropherotypes detected showed minor occurrence. Electropherotype 86/10-S1 persisted at low frequency through April 1987, and 85/7-L2 appeared intermittently during the nonepidemic months.

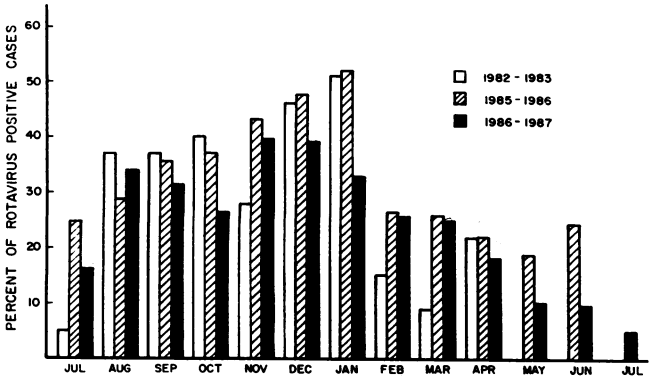


FIG. 2. Monthly distribution of rotavirus in pediatric diarrhea cases observed during July 1985 to July 1987 compared with the data on July 1982 to April 1983 reported previously by Wasi et al. (35).

**Occurrence of S electropherotypes during the three epidemics.** Rotaviruses with S electropherotypes occurred less frequently (57 of 393, 14.5%) than strains with L electropherotypes (336 of 393, 85.5%). S electropherotypes were detected during peak seasons in 1985 to 1986 and 1986 to

TABLE 2. Prevalence of rotavirus infection among diarrheic patients of different age groups, July 1985 to June 1986

| Age | No. tested | No. positive (%) |
|---|---|---|
| <6 mo | 439 | 102 (23) |
| 6–11 mo | 314 | 143 (46) |
| 12–23 mo | 167 | 59 (35) |
| 2–4 yr | 10 | 4 (40) |
| 5–15 yr | 11 | 1 (9) |
| Total | 941 | 309 (32.8) |

TABLE 3. Prevalence of rotavirus infection among diarrheic patients of different age groups, July 1985 to July 1987

| Age (mo.) | No. tested | No. positive (%) |
|---|---|---|
| <3 | 378 | 62 (16.4) |
| 4–6 | 335 | 106 (31.6) |
| 7–9 | 202 | 93 (46.0) |
| 10–12 | 130 | 43 (33.0) |
| 13–15 | 61 | 18 (29.5) |
| 16–18 | 54 | 17 (31.5) |
| 19–21 | 41 | 10 (24.4) |
| 22–24 | 19 | 5 (26.3) |
| Total | 1,220 | 354 (29.0) |

1987 but not during low seasons. By contrast, L electropherotypes occurred throughout the study period (Fig. 3).

Three rotavirus samples collected during the 1982 to 1983 epidemic were S electropherotypes (82/7-S1). They appeared in July, August, and November 1982. This electropherotype was not detected subsequently (Fig. 3). S electropherotypes during the 1985 to 1986 epidemic comprised 16.5% of the rotavirus samples analyzed (39 of 234). Two S electropherotypes, 85/7-S1 and 85/11-S2, were detected between August 1985 and May 1986. Only 85/7-S1 predominated (37 of 234, 15.8%), and it correlated well with the overall epidemic pattern (Fig. 3). The 85/11-S2 electropherotype was detected in only two patients in November 1985. During the 1986 to 1987 epidemic, the only S electropherotype was 86/10-S1 and it was the second most frequent electropherotype.

**Identification of subgroups and serotypes.** The 110 rotavirus strains electropherotyped in the above experiment were randomly selected as representative(s) of various electropherotypes, plus another 16 rotavirus samples were subgrouped and serotyped by ELISA using specific monoclonal antibodies. Serotype specificity or subgroup specificity or both could be identified directly from stool suspensions of 121 rotavirus strains (Table 4). One strain showed both subgroup I and II specificities, and this correlated with the PAGE result showing a mixed RNA migration pattern of both S and L electropherotypes (Fig. 4). Only five strains could not be subgrouped or serotyped, including one with an atypical electropherotype (Fig. 4).

**Correlation among subgroup, serotype specifities, and RNA electropherotypes.** Examination of all 110 rotavirus strains with known electropherotypes and antigenic specificities (Table 4) showed that all subgroup I strains had S RNA patterns. All 16 serotypable subgroup I rotaviruses were serotype 2. All 90 subgroup II strains (L electropherotypes) were serotype 1 (71 strains) and serotype 4 (9 strains). One strain identified as serotype 1 showed neither subgroup I nor II specificity. In the 97 strains studied, it was found that those with the same serotype could show different electropherotypes, but that those with identical electropherotypes always showed the same serotype (Table 4).

**Segment variations among serotypes.** Coelectrophoretic comparison of 22 rotavirus L electropherotypes revealed

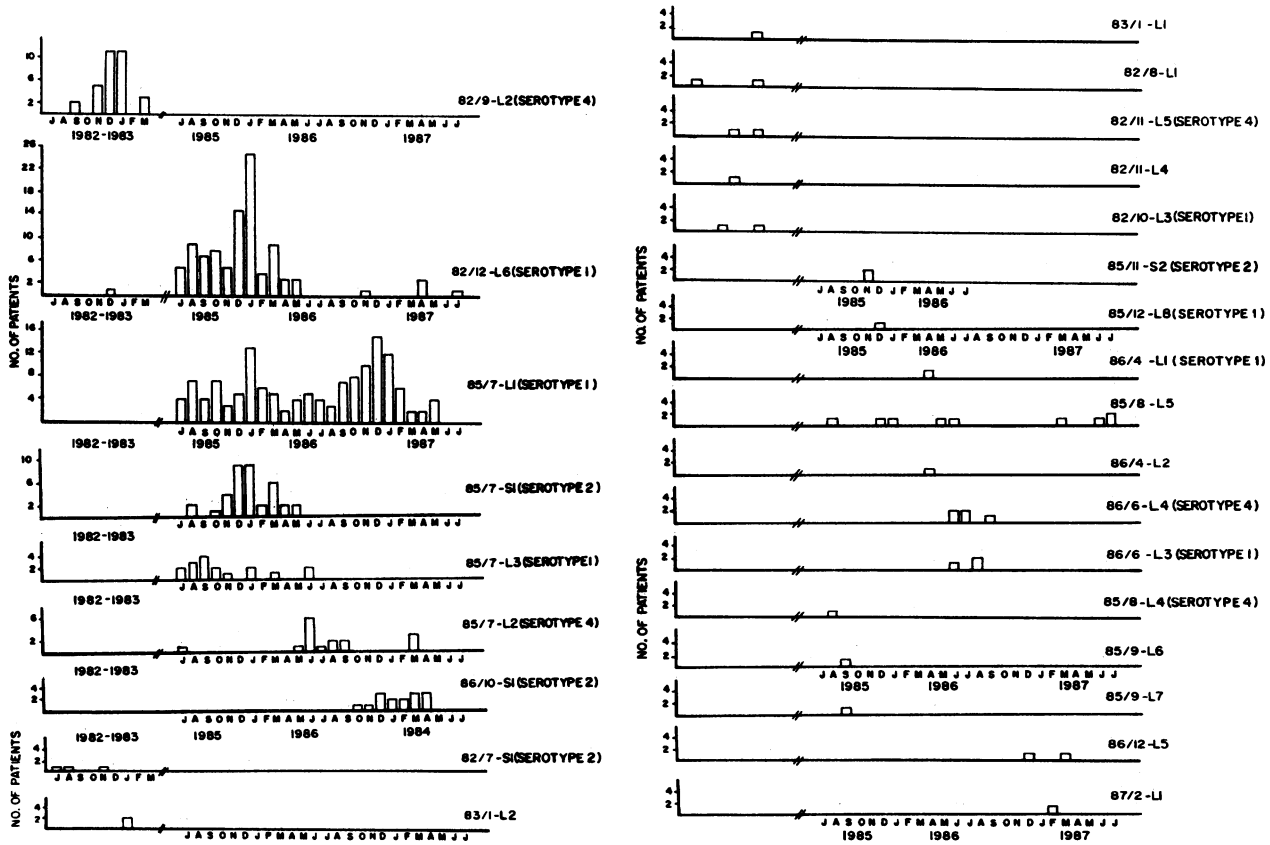


FIG. 3. Monthly distribution of individual human rotavirus electropherotypes in greater Bangkok during 1982 to 1983 (left) and 1985 to 1987 (right).

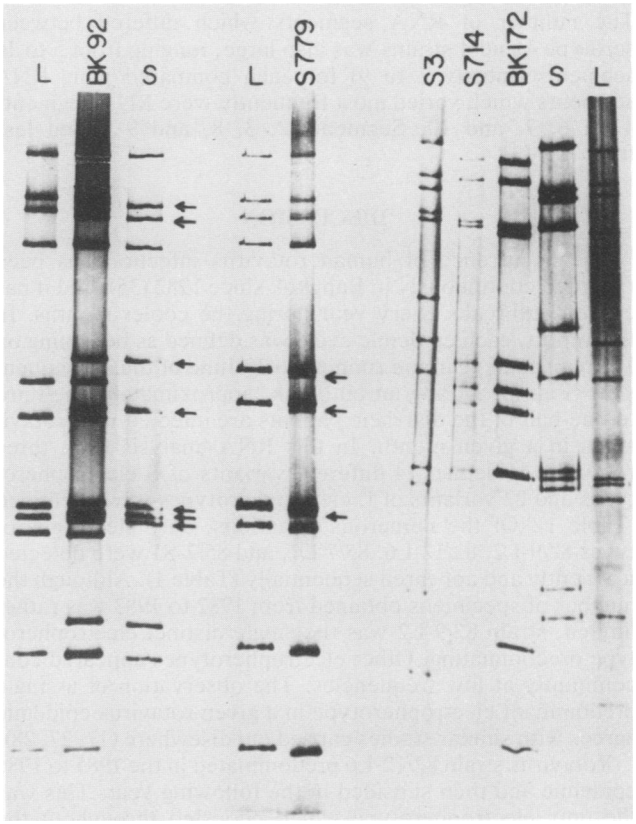


FIG. 4. RNA migration patterns of rotaviruses obtained from two patients with mixed infections. In one child, a mixture of an S and an L strain was found (BK92). In the other child, a mixture of two long strains was shown (S779). Arrows indicate the presence of extra RNA bands. Strains S3, S714, and BK172 showed atypical RNA migration patterns compared with standard S and L electropherotypes. Because the figure was compiled from portions of three different polyacrylamide gels, direct comparison among RNA patterns in this figure is not possible.

variations in the mobilities of all 11 RNA bands, but variation occurred most often with segments 4, 5, 6, 7, and 10; segments 2, 3, 8, and 9 showed less variation and segments 1 and 11 showed the least. Differences in compared pairs ranged from 1 to all 11 RNA segments.

Differences among the four S electropherotypes were observed in segments 2, 7, 8, 9, and 10, with the highest frequency in segment 8 and the lowest in segment 10. Segment differences in compared pairs ranged from two to four.

Comparison among strains of the same and of different serotypes showed variation in segments 3, 4, 5, 6, 7, and 10 among seven electropherotypes of rotavirus serotype 1 and in segments 2, 7, 8, and 9 among three electropherotypes of rotavirus serotype 2. The range of segment differences detected between pairs was one to five and two to four for serotypes 1 and 2, respectively. However, all 11 segments were shown to vary when five electropherotypes of rotavirus serotype 4 were compared, ranging from 1 to 11 but mostly 10.

Comparison of rotavirus strains of different serotypes showed differences in PAGE migration for most RNA segments. When strains of serotype 1 or 4 were compared with strains of serotype 2, 9 to 11 RNA segments were different.

TABLE 4. Subgroup and serotype identification and correlation with RNA electropherotype of human rotaviruses in 126 stool specimens collected from patients during 1982 to 1983 and 1985 to 1987

| Antigenic property | No. of samples tested | No. of strains with indicated electropherotype | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 82/7-S1 | 82/9-L2 | 82/10-L3 | 82/11/L5 | 82/12-L6 | 85/7-S1 | 85/7-L1 | 85/7-L2 | 85/7-L3 | 85/8-L4 | 85/11-S2 | 85/12-L8 | 86/4-L1 | 86/4-L2 | 86/6-L3 | 86/6-L4 | 86/10-S1 | |
| Subgroup 1 | | | | | | | | | | | | | | | | | | | |
| Serotype 2 | 16 | | | | | | 10 | | | | | 2 | | | | | | 4 | 16 |
| Nonserotypable | 4 | 1 | | | | | 2 | | | | | | | | | | | 1 | 4 |
| Subgroup II | | | | | | | | | | | | | | | | | | | |
| Serotype 1 | 75 | | | 1 | | 33 | | 33 | | 1 | | | 1 | 1 | | 1 | | | 71 |
| Serotype 4 | 13 | | 3 | | 1 | | | | 3 | | 1 | | | | | | 1 | | 9 |
| Unclassified | 10 | | | | | 4 | | 2 | | 2 | | | | | 1 | | | | 9 |
| Subgroups I and II | 1 | | | | | | | | | | | | | | | | | | |
| Unclassified subgroup | | | | | | | | | | | | | | | | | | | |
| Serotype 1 | 1 | | | | | 1 | | | | | | | | | | | | | 1 |
| Serotype 3 | 1 | | | | | | | | | | | | | | | | | | |
| Unclassified | 5 | | | | | | | | | | | | | | | | | | |

The number of RNA segments which differed between serotype 1 and 4 strains was also large, ranging from 5 to 10 segments (mostly 7 to 9) for each compared pair. RNA segments which varied most frequently were RNA segments 4, 5, 6, 7, and 10. Segments 2, 3, 8, and 9 varied less frequently.

## DISCUSSION

The occurrence of human rotavirus infection has been recorded continuously in Bangkok since 1982 (35), and it has caused outbreaks every year during the cooler months. In this report, each epidemic cycle was defined as beginning on 1 July of each year and running to 30 June of the subsequent year (Fig. 2). During an outbreak, approximately one-third to one-half of the diarrheic patients are infected with rotaviruses in a given month. In this RNA analysis from three rotavirus epidemics, 4 different variants of S electropherotypes and 22 variants of L electropherotypes were identified (Table 1). Of the numerous variations, only electropherotypes 82/9-L2, 82/12-L6, 85/7-L1, and 85/7-S1 were detected frequently and appeared sequentially (Table 1). Although the number of specimens obtained from 1982 to 1983 was rather limited, strain 82/9-L2 was the single distinct electropherotype predominating. Other electropherotypes appeared concomitantly at low frequencies. The observation of a single predominant electropherotype in a given rotavirus epidemic agrees with similar studies carried out elsewhere (17, 27, 28).

Rotavirus strain 82/12-L6 predominated in the 1985 to 1986 epidemic and then subsided in the following year. This was the only electropherotype which persisted throughout the study period. Previous studies have shown that a single distinct electropherotype can predominate in a community through successive years (25, 27). Unfortunately, rotavirus strains collected from April 1983 to June 1985 were not available for electropherotyping. Thus, the role of strain 82/12-L6 during that period is not known. Despite the limited numbers of specimens and the limited time period of this study, we confirm a previous statement (30) that a strain may be detected infrequently one season but become the predominant strain in the next or it may become the predominant strain without being previously detected. However, it must be noted that these studies included only hospitalized patients and therefore would miss strains that may have circulated in the community but caused mild illnesses during a period of substantial group immunity only to emerge later as a predominant cause of serious diarrhea.

Electropherotype 85/7-L1 predominated for two successive rotavirus seasons, although it shared predominance with 82/12-L6 during the 1985 to 1986 season. We are continuing to observe the etiological role of this rotavirus strain in subsequent outbreaks.

S electropherotypes were demonstrated in every rotavirus outbreak in this study, but the total incidence was much lower than that for L electropherotypes, as has been observed generally (10, 28, 36). Although their monthly incidence increased (20 to 40% during the peak season), they never became predominant (data not shown). An epidemic of rotavirus gastroenteritis caused by a single S electropherotype in Papua, New Guinea, has been reported (1). A parallel study in Lampang province in Thailand revealed that the S electropherotype predominated during a 1987 to 1988 outbreak (unpublished data). Shifts of the S electropherotype occurred in every outbreak in the present study.

The sequential appearance of several electropherotypes during the short period of this study illustrated the rapid change in the local population of rotaviruses, suggesting progressive alteration of the viral genome within the community. Possible mechanisms include modification of the viral genome and generation of genetic diversity through genomic reassortment in vivo (6). Mixed infections by different types of rotaviruses are a prerequisite for the latter mechanism, and such infections were indeed observed in our study. Specimens from two patients revealed more than 11 genome segments, suggesting simultaneous infection by more than one strain. Alternatively, modifications in the length of the RNA segments could have occurred during infection. One sample collected from a girl aged 3 months in November 1985 had a mixture of S and L electropherotypes (Fig. 4) and both subgroup I and II specificities. Only the L electropherotype could be isolated and identified as subgroup I, serotype 1, which was found circulating in the community at the time this mixed infection was observed. A specimen collected from a second child (girl, aged 2 years) in May 1986 showed 12 genome segments (Fig. 4) which could have resulted from a simultaneous infection by more than one L electropherotype. The RNA pattern resembled that of 85/7-L2 mixed with another strain that resembled none of the electropherotypes detected in this study. The rotavirus from this fecal specimen was neither isolated nor characterized for its antigenic properties. Several studies (21, 25, 29) have reported the presence of mixed rotavirus electropherotypes in diarrheic patients. The evidence suggests that the interaction between different electropherotypes in nature is significant and the epidemiological conditions which would support and maintain such interactions are important. A high human population density and a large group of susceptible individuals may facilitate transmission of rotaviruses and provide ample opportunities for mixed infections of different rotavirus strains.

Antigenic diversity exists among rotaviruses, and this has important implications for diagnosis, epidemiology, and vaccination strategies. Recently, the enzyme immunoassay technique has been developed for subgrouping and serotyping human rotaviruses using highly specific monoclonal antibodies prepared against each serotype or against subgroup antigens (5, 23). Most of our tested specimens (105 of 126, 83.3%) were successfully identified for both subgroup and serotype specificities (Table 4). Subgroup or serotype antigens alone were identified in 17 samples (13.5%). The inability to type the remaining five samples (4%) might have resulted from an insufficient amount of rotavirus antigen in the stool suspensions, as has occurred in other studies (19, 32), or from the lack in the rotavirus strains studied of epitopes recognizable by the monoclonal antibodies of the test system. One of these unidentified rotavirus samples was shown to be atypical by RNA PAGE analysis and has proven to be a group C rotavirus (24). Atypical rotaviruses were detected at low frequency (two cases in 1986 and one case in 1987). Rotaviruses with unusual antigenic properties should not be excluded. Recently a fifth (22) and a sixth (4) serotype of human rotaviruses and nonsubgroup I or II rotaviruses have been reported (13).

Previous reports on association between subgroup specificity and RNA segment 10 and 11 migration (7, 15) were confirmed in our study. There are reports, however, of subgroup I exhibiting an L electropherotype and subgroup II exhibiting an S electropherotype (3, 31). Additional investigation is necessary.

Another observation similar to previous findings (23) is that all rotavirus strains with the identical electropherotype show the same serotype specificity. However, within the

same serotype, different electropherotypes may be found, so that genomic change may not result in a concurrent serotypic change. Although electropherotype alone cannot be used to identify the serotype of a virus isolate (2), the serological and molecular techniques may very well complement one another in evaluating naturally occurring rotavirus alterations and in characterizing virus strains in outbreaks. The detection of new rotavirus electropherotypes may be used to predict the circulation of a different serotype that could play an important role in subsequent outbreaks. Such information is necessary for the prevention of rotavirus infections and for the development of an efficient vaccine. Uhnoo and Svensson (33) have suggested that shifts in electropherotype indicate an antigenic change that may reflect a change in the immunity of the population at risk. In the present study, we know that a serotype 4 rotavirus (82/9-L2) predominated during the 1982 to 1983 epidemic in greater Bangkok and that one appeared again (85/7-L2) at a low level during the low season after the 1985 to 1986 epidemic (Fig. 3). This might have resulted from the induction of a group immunity in the population by the epidemic spread of rotavirus serotype 4 during the 1982 to 1983 period. Serotypes 1 (82/10-L3 and 82/12-L6) and 2 (82/7-S1) occurred sporadically during that time, and both became major rotaviruses in the 1985 to 1986 (82/12-L6, 85/8-L1, 85/7-L3, and 85/7-S1) and 1986 to 1987 (85-7-L1 and 86/10-S1) epidemics. These data agree with the previous findings by Rodger et al. (25) which showed that causal rotavirus serotypes could persist for two continuous epidemic seasons before they declined or disappeared. The epidemiological features of the rotavirus infections in Bangkok appear similar to those observed in European countries (11) and in Brazil (20), in which serotype 1 strains were most frequent followed by serotypes 2 and 4. Serotype 3 was the least frequent. Since we found major shifts in frequency from one serotype to another during consecutive years of the study but found only one case of rotavirus reinfection, it appears that children develop cross-protective immunity after infections.

Detailed electropherotype analysis revealed that RNA groups I and III showed the greatest diversity (Fig. 1) and that more variation occurred with L than with S electropherotypes. It could be explained from pure logic that RNA groups with more segments (I and III) and electropherotypes with more space (L) allow for more possible patterns in PAGE. Comparison among strains of the same serotype revealed that RNA segments of serotype 1 strains seemed to be more stable than those of serotype 4. There was no correlation, however, between serotype specificity and the mobility of any specific genomic segment.

This study has shown that most children hospitalized for diarrhea are under 12 months old and that rotavirus diarrhea is associated with children under 2 years old. Thus, research on rotavirus should emphasize children under 2 years old. The rate of rotavirus infection peaked at 7 to 9 months of age and was low in diarrheic infants under 3 months old. Passive acquired immunity against rotavirus infection might be strong enough to protect infants under 3 months old and then decline in older age groups. If a vaccination program were to be used to reduce rotavirus morbidity in this situation, it would have to be carried out very early during infancy. A more detailed study of resistance to rotavirus-induced illness in infants under 3 months old may provide useful information for designing a logical schedule for rotavirus vaccination.

A correlation between human rotavirus serotype or electropherotype and patient age was not evident in this study. It did not appear that any particular strain was significantly more prevalent in any particular age group.

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