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Temporal Variation of Malaysian Rotavirus Electropherotypes

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An analysis of rotavirus electropherotypes circulating in Kuala Lumpur, Malaysia, over 7 years showed that all except 1 of the 360 electropherotypes encountered were characteristic of group A rotaviruses. The long electropherotype predominated annually, and there was a rarity of short electropherotypes. Extensive genome variability and cocirculation of different electropherotypes were observed annually. A sequential appearance of the predominant electropherotype was observed in all years of the study, except for 1985 and 1988, when one electropherotype predominated throughout the study periods. There was no shift in the predominant electropherotype over a 6-year period.

Rotaviruses are a major cause of acute gastroenteritis in children globally (6). The virus genome consists of 11 segments of double-stranded RNA separable by polyacrylamide gel electrophoresis to yield electrophoretic profiles, termed electropherotypes, which exhibit extensive diversity. Simultaneous coexistence of viruses with a number of different electropherotypes, a sequential pattern of appearance of electropherotypes, and a predominance of one electropherotype each year are features associated with rotavirus molecular epidemiology (5).

The majority of electrophoretic studies of rotaviruses either analyzed relatively few samples or examined samples for a limited time period (5). In this paper, we describe an analysis of rotavirus electropherotypes circulating in Kuala Lumpur, Malaysia, for 7 of the years between 1978 and 1988. This report also represents the first documented description of Malaysian human rotavirus electropherotypes. Although the prevalence of rotavirus gastroenteritis has been investigated in Malaysia previously (11, 12), there have been no reports on the electropherotypes circulating in the population.

Stool specimens were obtained from children up to the age of 6 years that had been admitted with acute gastroenteritis to the University Hospital and General Hospital, Kuala Lumpur, Malaysia. RNA was extracted directly from the specimens with phenol-chloroform by the method used in the laboratory of I. Holmes (Melbourne, Australia) and electrophoresed in 3% polyacrylamide gels at 7.5 mA for 16 to 18 h. The gels were stained with silver nitrate and photographed without a filter. Electropherotypes were determined in comparison to the bovine (U.K.) or simian (SA11) rotavirus electropherotypes.

Table 1 gives the temporal distribution of the incidence of rotavirus-positive gastroenteritis in each year of study. Specimens for 1978, 1983, and 1984 constituted a retrospective analysis, thus their smaller sample sizes. Specimens for 1985 represented a full-year analysis, whereas those for 1986 to 1988 were for approximately half of each year. The higher incidence of rotavirus-positive gastroenteritis in 1978, 1983, and 1984 could be a result of the smaller sample sizes, which may have led to an overestimate. The full-year study of 1985 yielded an incidence of 19%, and the half-year studies between 1986 and 1988 yielded values between 5.7 and 19.3%. The reason for the variability is not clear, and it does not appear to be due to differences in sample size or specimen collection period.

All the electropherotypes encountered in the study, except one, were typical (group A) rotavirus electropherotypes. Only one atypical electrophoretic profile was encountered (N. Rasool, unpublished data). Among the 359 typical profiles, 2 mixed electropherotypes were also observed giving frequencies of 0.05 and 0.1%, respectively, for the detection of atypical and mixed profiles.

Table 2 gives the frequencies of the major group A rotavirus electropherotypes encountered in each year of study. To facilitate their analysis, the classification system adopted by Tam et al. (10) was used. In this sytem, the long electrophoretic pattern was designated II and the short pattern was designated I. RNA patterns I and II were further subclassified according to the resolution of the RNA segments. When all segments were resolved, the pattern was referred to as A; B denotes comigration of segments 2 and 3, C denotes comigration of segments 7 and 8; and D denotes comigration of segments 8 and 9. We designated comigration of segments 7 to 9 as G.

We encountered only three major short and three major long electropherotypes over the 7-year period. Short rotavirus electropherotypes were a rarity in each year of study and the majority (96%) of the electropherotypes encountered were of the long type (Table 2). Except for 1978, the IIC electropherotype predominated in each year of study. The IIA electropherotype was the next most frequently encounted electropherotype. The IIG electropherotype was also a rarity. A cocirculation of more than one electropherotype was observed annually.

Figure 1 shows the RNA profiles of the major electro-

TABLE 1. Temporal distribution of incidence of rotavirus-positive gastroenteritis in hospitalized children in Kuala Lumpur[a]

Yr	No. tested	No. positive	Incidence (%)
1978	21	7	33.3
1983	18	8	44.4
1984	31	26	83.8
1985	879	167	19.0
1986	247	14	5.7
1987	407	48	11.8
1988	467	90	19.3

[a] Presence of rotavirus-positive gastroenteritis was tested by polyacrylamide gel electrophoresis of RNA extracted from stool specimens.

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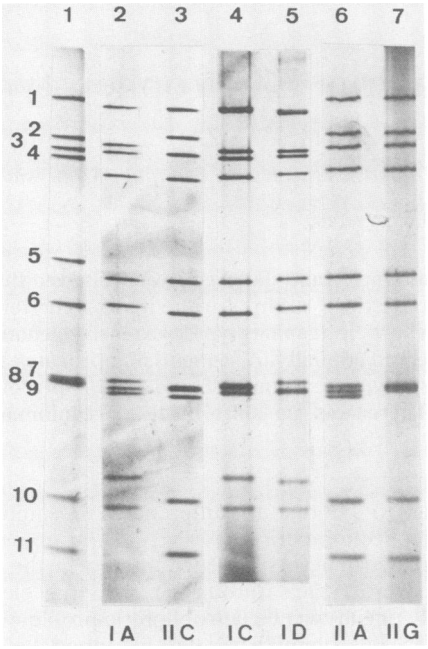

FIG. 1. RNA profiles of major electropherotypes. The classification type (see text) of the major electropherotype is shown at the bottom of the gel. The positions of RNA segment bands in lane 1 are shown to the left of the gel. Lanes 1 through 3, lanes 4 and 5, and lanes 6 and 7 were from different gels. Lanes: 1, bovine U.K. rotavirus; 2, G35; 3, G28; 4, U7; 5, H528; 6, F23; 7, F15.

pherotypes. The most apparent variation in electrophoretic mobility within RNA patterns I and II was observed for genome segments 7 to 9. Segments 2 and 3 of the short electropherotypes appeared to migrate closer together than those of the long electropherotypes. Between and within the major electropherotypes, minor variations in the electrophoretic mobilities of the other RNA segments were also observed (Fig. 1 and 2).

TABLE 2. Frequency of group A rotavirus electropherotypes[a]

Electropherotype	Frequency of electropherotype in:							
	1978	1983	1984	1985	1986	1987	1988	All yr studied (% of total)
IA				7		1		8 (2.3)
IC		1		1	4			6 (1.7)
ID							1	1 (0.3)
IIA	5		8	45	4	14	13	89 (25.4)
IIC	2	6	16	113	6	30	73	246 (69.5)
IIG			2	1		1		4 (1.1)

[a] A total of 354 electropherotypes, not including those of mixed, atypical, and three depleted specimens, were analyzed. The predominant electropherotype and frequency (percentage of electropherotypes encountered each year) by year were as follows: 1978, IIA (71%), 1983, IIC (86%); 1984, IIC (62%); 1985, IIC (68%); 1986, IIC (43%); 1987, IIC (65%); 1988, IIC (84%).

Figure 2 shows the sequential appearance of electropherotypes between January and August 1984. It is observed that there was no clear sequence of appearance of different electropherotypes. This was also true for the other years of study. The IIC electropherotype appeared to predominate between January and April 1984, whereas the IIA electropherotype predominated between May and August. The IIC electropherotype predominated in all months of 1985 (R. Y. Othman et al., manuscript in preparation). In 1987, the IIA electropherotype predominated in August, whereas the IIC electropherotype predominated between September and December (data not shown). In 1988, as in 1985, the IIC electropherotype predominated throughout the study period (data not shown). The sample sizes for 1978 and 1983 were too small for such determinations to be made, whereas the dates of sample collection in 1986 were not recorded.

As demonstrated elsewhere in the world (5), two major RNA patterns designated I (short pattern) and II (long pattern) could be distinguished in Kuala Lumpur, with the former constituting a minority (4%) of all electropherotypes encountered. This compares with an incidence of 11% in a

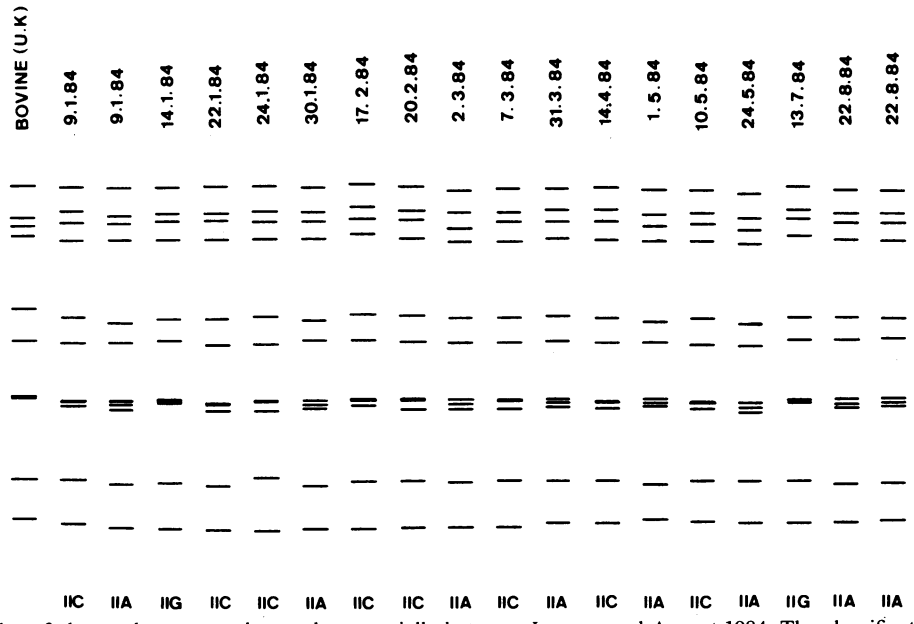

FIG. 2. RNA profiles of electropherotypes observed sequentially between January and August 1984. The classification type (see text) of the major electropherotype is shown at the bottom. The date that the sample was collected is shown at the top.

study by Espejo et al. (3) in Mexico City. A minority of short electropherotypes appears to be the norm globally (5). The data of Espejo et al. (1–4) were also the first to suggest a shift in the predominant electropherotype in sequential years. Our data suggest a shift in the predominant electropherotype between 1978 (IIA) and the subsequent years of study (IIC). However, the predominance of the IIA electropherotype in 1978 could also be an error resulting from the small sample size (Table 2).

The largest epidemiological study thus far was reported in Melbourne, Australia (8); it also examined human rotaviruses over a 7-year period (1973 to 1979) by RNA electrophoresis. The study analyzed small sample numbers per year and demonstrated extensive genome variability, the simultaneous coexistence of viruses with a number of different electropherotypes, and a sequential pattern of electropherotype appearance. Our present analysis confirms these observations, except that in 1985 and 1988, the IIC electropherotype predominated throughout the study period. As observed in Melbourne, one electropherotype predominated each year in Kuala Lumpur, in combination with less-common types. The Melbourne study, however, suggested that a major shift in the prevalent phenotype may occur every 2 to 3 years. This was not the case in Kuala Lumpur, where the IIC electropherotype predominated for 6 consecutive years (1983 to 1988).

Most studies that have examined a reasonable number of stool specimens have reported the presence of mixed rotavirus electropherotypes (7, 9), which suggest the possibility of genetic reassortment in an individual infected with two or more viruses. Our data suggest a low frequency of mixed infections (0.6%) among rotavirus-positive cases and an even lower frequency (0.1%) of their detection. Therefore, genetic reassortment of the type discussed above might be a rare event, if any, under natural conditions.

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