Rotavirus electropherotypes from the Kuala Lumpur Hospital: a re-examination after an interval of seven years

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Abstract

The objective of this study was to ascertain the extent changes have occurred in the epidemiology of human rotavirus electropherotypes from the same location 7 to 8 years after an earlier study. Genomic RNA profiles of rotaviruses from diarrhoeic children admitted to the Kuala Lumpur Hospital from April to December 1996 were determined by polyacrylamide gel electrophoresis and silver staining. A total of 179 group A rotaviruses were detected from 870 children: 175 with legible staining of all RNA segments were classified into 14 distinct electropherotypes (10 and 4 with long and short migration patterns respectively). In addition, the results revealed: high predominance of long pattern electropherotypes (94% of the total electropherotypes); most long electropherotypes with RNA profiles which all 11 RNAs migrated separately (8 of 10 electropherotypes); all short electropherotypes had segments 2 and 3 that co-migrated; presence of a very numerically dominant electropherotype (75% of all electropherotypes); frequent co-circulation of the dominant electropherotype-present throughout the study period-with other electropherotypes present for limited periods; sequential temporal appearances by similar electropherotypes. These observations were similar to that of an earlier study conducted in 1988189. Nevertheless, the dominant electropherotype in the present study was different and not among the electropherotypes detected in the earlier study.

Key words: human rotavirus, electropherotypes

INTRODUCTION

Rotaviruses are an important cause of gastroenteritis in human and a variety of animals.' Rotaviruses are classified within the family Reoviridae in the genus *Rotavirus*.[2] The viruses are differentiated serologically into groups, subgroups (for only group A rotaviruses) and serotypes.[3] Besides serological classifications, the genome of rotavirus which consists of 11 segments of double-stranded (ds) RNA can be separated into distinct bands by polyacrylamide gel electrophoresis (PAGE) and visualised by ethidium bromide or silver staining.[4,5] The overall pattern produced is known as the viral electropherotype and is a reproducible trait of a virus strain. Thus each distinct RNA profiles defined a different viral electropherotype or strain. Electropherotypes are not used in formal virus taxonomy to classify rotaviruses into different serotypes and subgroups because corresponding segments of rotaviruses with different genome composition can co-migrate[6"], and co-migrating RNA segments of different electropherotypes can code for proteins with different serotypic specificities.[8] Nevertheless, electropherotypes are useful epidemiological indicators on the evolution and spread of rotavirus strains, and the observation of changes in electropherotypes helps us monitor the patterns of disease outbreaks and transmission.[9-11] Electropherotyping of rotaviruses from dianhoeic children from the Kuala Lumpur Hospital was done by us from November 1988 to October 1989.[12] As a relatively long period has passed, it was of interest to determine the extent rotavirus electropherotypes from the same location has changed as viruses are inherently variable and genetic changes of rotavirus can arise by antigenic shift and drift, reassortment and rearrangement.[8] Thus in 1996, epidemiological typing of rotavirus by analysis of genomic RNAs using PAGE was performed with exactly the same procedures used in the earlier study .

MATERIALS AND METHODS

Faecal samples

Faecal samples were collected from 870 diarrhoeic children from the Kuala Lumpur Hospital from April to December 1996. The samples were stored frozen until they were tested.

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Rotavirus detection

The procedures used for viral dsRNA extraction from faecal samples, PAGE and silver staining (based on Herring *et al.,* [4] and Roger and Holmes[13]) were exactly the same as in the earlier study.[12] Briefly, double-stranded RNAs were extracted from faeces using a chloroform-phenol mixture and then electrophoresed using Laemmli's discontinuous PAGE system[14] without sodium dodecyl sulfate in all buffers. Electrophoresis was done at 8°C using a 10 cm long **7%** separating gel with a **3%** stacking gel and a current of 20 mA per slab gel for the first hour followed by 10mA for the next 18 hours. After electrophoresis, the gels were stained with silver nitrate. All samples were screened using 20 μl sample volume and negative samples were retested using 80 μl.

Labelling of rotavirus electropherotypes

Rotaviruses with 'long' RNA profiles due to the fast migrating RNA segments 10 and 11 and 'short' RNA profiles due to the slower migrating segments 10 and were labelled 'L' and 'S' respectively. Both were followed by a number that identified a particular rotavirus electropherotype. Minor differences in RNA profile within each distinct electropherotype were denoted by a lower case alphabet after the number.

RESULTS

Rotavirus electropherotypes

All electropherotypes were group A rotaviruses based on the characteristic 4-2-3-2 distribution pattern of the genomic RNAs. Fig. 1 (lane 1-14 from the left) shows the 14 distinct electropherotypes detected in 9 months: 10 with 'long' RNA profiles (long electropherotypes: L1 - L10) and 4 with 'short' RNA profiles (short electropherotypes: S1-S4).

A basic variance in RNA profiles among the electropherotypes was the separate migration of all 11 RNA segments or co-migration of certain RNA segments. The former was more common among the long electropherotypes whereas all short electropherotypes have the latter profile with
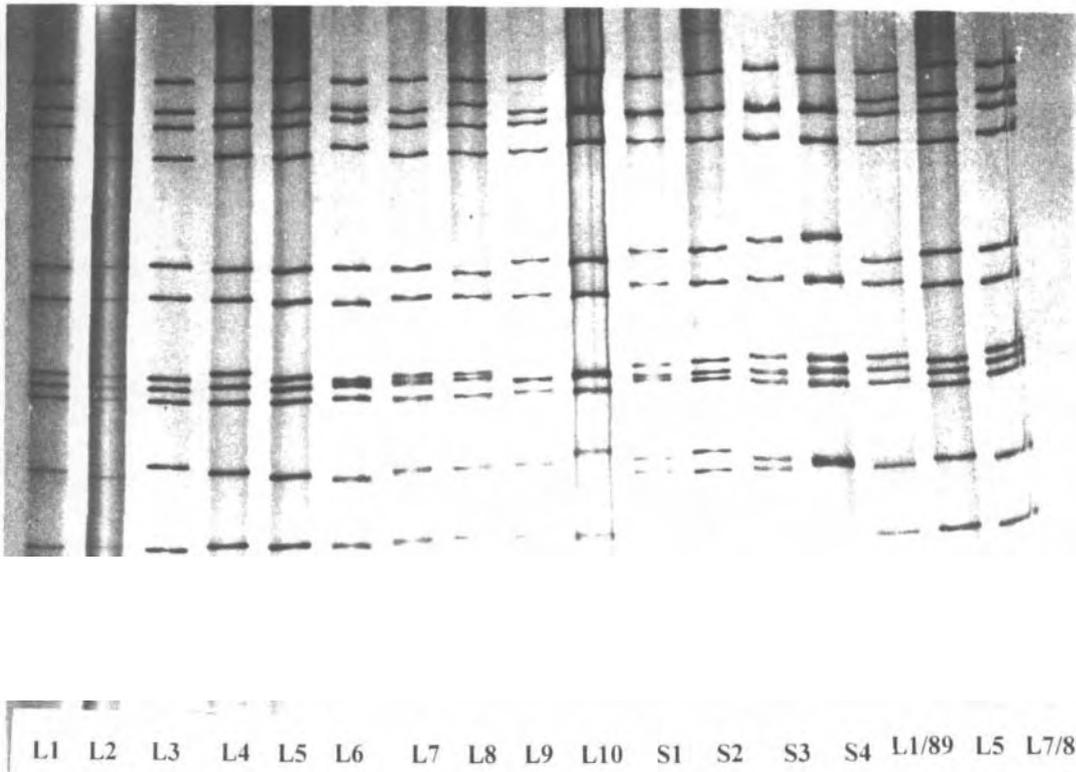

| L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 | L10 | S1 | S2 | S3 | S4 | L1/89 | L5 | L7/89 |

FIG.1: RNA profiles of different rotavirus electropherotypes detected from children admitted to the Kuala Lumpur Hospital.
Lane 1 (from the left) to 10: long electropherotypes L1 to L10; lane 11 to 14: short electropherotypes S1 to S4; lane 15: dominant electropherotype (L1/89) for 1988189; lane 16: dominant electropherotype (L5) for 1996; lane 17: electropherotype L7/89 from 1989.

co-migration of segments 2 and **3.** Besides this basic difference, there were other distinctive differences among different electropherotypes. In long electropherotypes, segments 2 and **3** of the large RNA segments 1-4 varied the most in relative migration positions that ranged from far apart to together. In contrast, segments 1 and 4 have basically the same mobility, or position in the gel, except for the slower migration of segment 4 of L6. The mobility of segments 5 and 6 was essentially similar in all long electropherotypes except one (L8) that has a faster migrating segment 5. The closely running triplet of segments 7, 8 and 9 has the most number of variable patterns: the different positions of segment 8 relative to 7 produced migration patterns in which segment 8 was located equidistant to segments 7 and 9 and progressively closer to segment 7 until both co-migrated. Segment 10 showed a continuum of positions while segment 11 occupied rather similar positions in all electropherotypes.

Among short electropherotypes (Fig. 1: lane 11-14), the mobility of RNA segments 1 to 4 was similar in **3** of the 4 electropherotypes while in S4, segment 4 migrated slightly faster. Segments 5 and 6 have **3** different migration patterns. The migration patterns of the triplet segments 7, 8 and 9, and the small segments 10 and 11 were different in all 4 electropherotypes. Among all electropherotypes, short and long, S4 was the only one in which segments 10 and 11 co-migrated.
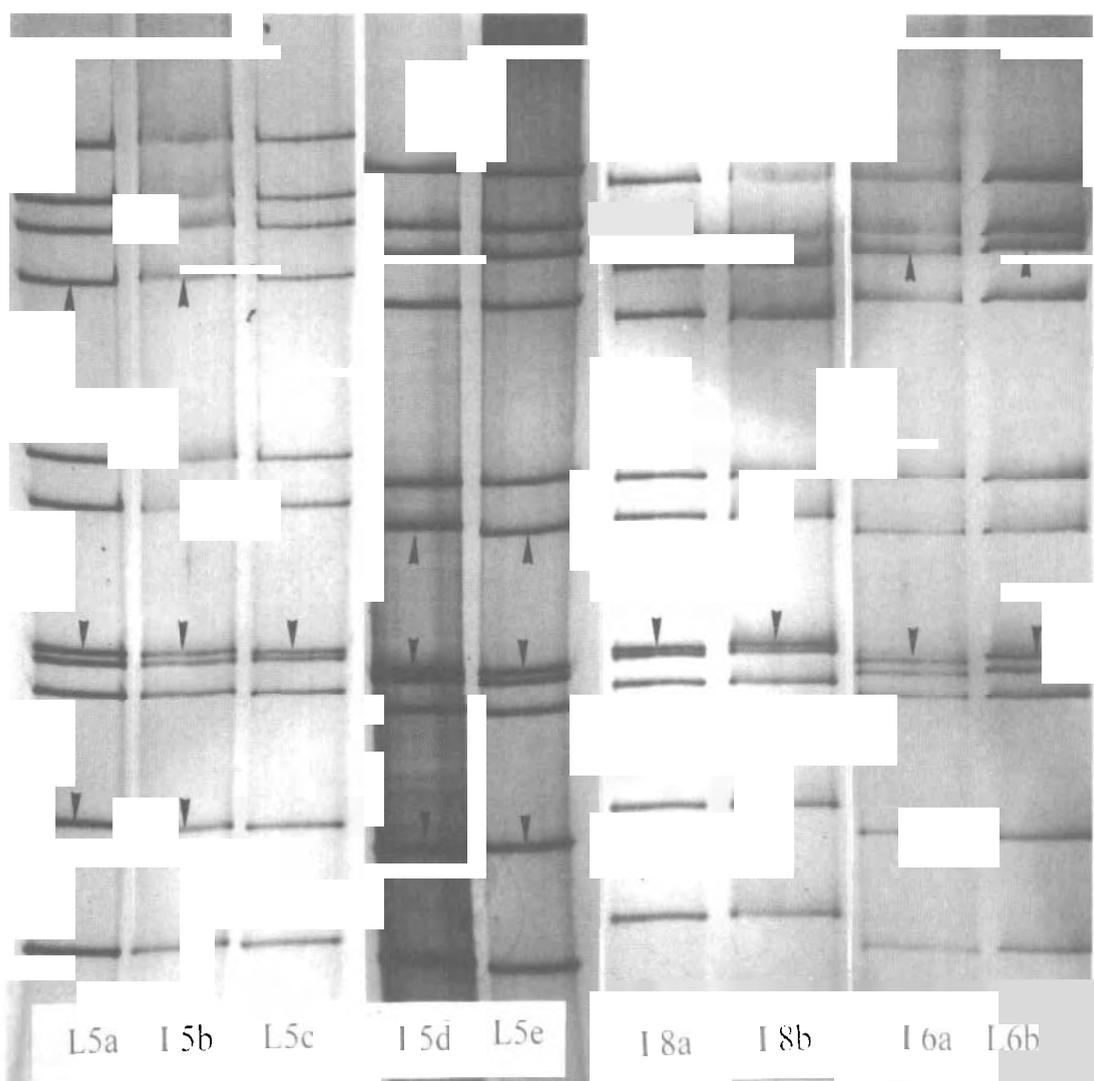

FIG. 2: RNA profiles of electropherotypes L5, 6 and 8 (composite of 4 different gels aligned at the top edges of the running gels) showing minor differences in migration of RNA segments within designated electropherotypes. Arrow heads indicate minor differences in mobility of particular RNA segments.

Minor differences within electropherotypes

Besides major differences in RNA profiles which separated the rotaviruses into distinct electropherotypes, minor but apparent variations were detected in 3 of the 8 electropherotypes which have more than 1 member (Fig. 2). All of them (L5,6,8), have small differences in mobility of segments 7 and 8. In addition, minor differences were also detected in other segments: segments 3 (L6a and L6b), 4 (L5a and L5b), 6 (L5d and L5e) and 10 (L5a and L5b, L5d and L5e).

Comparison of RNA profile of the dominant electropherotype for the year 1996 and 1988189

The overall numerically dominant electropherotypes in 1996 (Fig. 1, lane 16) and 1988/89 (Fig. 1, lane 15) have long migration patterns in which all 11 RNA segments migrated separately. However, their RNA profiles were dissimilar; the migration positions of segments 4, 5, 6, 7, 9 and 10 were clearly different. The dominant strain in this study has a RNA profile rather similar to an electropherotype (L7/89: Fig. 1, lane 17) present as a single member in 1989 but co-electrophoresis revealed that they were different (results not shown).

Numerical and temporal distributions of electropherotypes

Table 1 shows the numerical and temporal distribution, and co-circulation of different electropherotypes. There was a very numerically dominant electropherotype (L5). In comparison, the second most abundant electropherotype, L4, has 11-fold less members. Six of the 14 electropherotypes (43%) were detected only once.

Unlike the overall dominant electropherotype which was present for the entire period of study, others with more than 1 member were limited in the length of their appearances. In one (S2), all members appeared together in one month. Others were spread out either continuously over several months or in 2 separate periods of 1 to 3 months with intervals of 2 to 6 months between appearances.

L5 was the most common electropherotype in 7 of the 9 months. In November, it was co-dominant with the only other electropherotype (with a short migration pattern) detected and in the following month the dominant electropherotype was a short electropherotype detected only in that month.

TABLE 1: Numerical and monthly distribution of rotavirus electropherotypes

Electropherotype		No. of each electropherotype present in:								
Type	No. (%)	April	May	June	July	Aug	Sept	Oct	Nov	Dec
L1	3 (1.7)	1	1							1
L2	1 (0.6)				1					
L3	3 (1.7)	1	2							
L4	13 (7.4)	2	6	4	1					
L5	131 (74.8)	53	9	10	9	12	22	11	2	3
L6	5 (2.9)	2	1	1						1
L7	1 (0.6)	1								
L8	6 (3.4)	1	1	2						2
L9	1 (0.6)		1							
L10	1 (0.6)									1
S1	1 (0.5)				1					
S2	5 (2.9)									5
S3	1 (0.6)				1					
S4	3 (1.7)					1			2	
No. of different electropherotypes/ month		7	7	5	4	2	1	1	2	6

Co-circulation of electropherotypes was common but there was a temporal distribution in the number of circulating electropherotypes. It was more extensive in April to July with a total of 5-7 electropherotypes present monthly. In contrast co-circulation was absent in the following 2 months and involved just 2 electropherotypes in the following month. In December, it again increased to the level of April to July.

DISCUSSION

Comparison of rotavirus electropherotypes-either from different laboratories or the same laboratory over different periods of time-is only meaningful when the same conditions of gel electrophoresis are used as different conditions can result in different RNA migration patterns from the same virus.[15] This study which re-examined human rotavirus electropherotypes from the same source 7 to 8 years later was performed using the same PAGE procedure as before. In addition, the final analysis that defined different electropherotypes was performed by the same person.

Comparison of data on human rotavirus electropherotypes detected in 1996 with those obtained from rotaviruses detected 7 to 8 years ago[12] revealed many similarities. In this regard, long electropherotypes continued to be very prevalent: accounting for 94% of the rotaviruses detected in 1996, close to the 92% of 1988189. Genetic variability, as expressed by number of distinct electropherotypes, remained high and at rather similar levels with 10 long and 4 short electropherotypes detected in 1996 compared with 12 and 5 in 1988189. In both periods a very numerically dominant electropherotype was present: representing 79 and 75% of the total number of long electropherotypes and all rotaviruses detected respectively in 1996 compared with figures of 79% and 72% in 1988189. Likewise, the proportion of the dominant electropherotype among the short electropherotypes was also rather similar (1996: 50%. 1988189: 58%). Most long electropherotypes continued to have RNA profiles in which all 11 genomic segments migrated separately (1996: 8 of 10 electropherotypes, 1988189: 8 of 12) and all short electropherotypes have segments 2 and 3 that co-migrated. Co-circulation of different electropherotypes remained a common event occurring in 7 of the 9 months in 1996 and in all the 8 corresponding months in 1988/89 when rotavirus was detected. The range of the number of co-circulating electropherotypes

present each month was essentially similar; from 7-2 in 1996 and 7-3 in 1988189. Whether the similarities detected in the 2 different periods reflected a situation that remained essentially the same over the years or were coincidental occurrences is unknown.

A notable difference in 1996 compared with the situation 7 to 8 years ago was the change in the dominant electropherotype. This particular electropherotypes was not present in the earlier period.

Minor differences in the migration of 1 or more RNA segments were observed among members of some electropherotypes. Numerical superiority of an assigned electropherotype was not the sole factor for the emergent of minor differences as they also occurred in electropherotypes with less members. Nevertheless, most minor variations were detected in the numerically dominant strain. Assignment of rotaviruses into discreet electropherotypes based on their RNA migration profiles was more subjective in cases when continuum of small changes in RNA mobility were observed. Although there were clearly minor differences among members grouped together in a defined electropherotype, the epidemiological importance such minor changes is moot.

ACKNOWLEDGEMENTS

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