

## Molecular epidemiology of human rotavirus

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### SUMMARY

The epidemiology of human rotavirus (HRV) in north-east Scotland was investigated between 1982 and 1984. During this period 708 HRV infections were recorded. The majority (83%) of the infections were in children less than 5 years of age although some were recorded in adults. The peak incidence occurred in the winter months although a high number of HRV infections was reported during the summer of 1983. A total of 840 faecal specimens containing rotavirus were screened for HRV genome RNA by polyacrylamide gel electrophoresis. Seven hundred (83%) specimens gave RNA profiles suitable for establishing the HRV electrophoretype. Twenty-five different electrophoretotypes were identified, of which 21 had 'long' RNA profiles and four had 'short' RNA profiles. There was extensive co-circulation of distinct electrophoretotypes although during any one epidemic period the majority of viruses belonged to a relatively small number of types. Comparison of viruses collected from hospitalized and non-hospitalized patients showed no differences in electrophoretype distribution. HRV was identified in faecal specimens from a wide age group and no correlation was demonstrated between age of patient and electrophoretype of the infecting virus.

### INTRODUCTION

Human rotavirus (HRV) is a major cause of gastroenteritis in young children (in the age group 6 months to 5 years) (Bryden *et al.* 1975). HRV infections in adults are generally mild but in the elderly (age group 60–90 years) severe infections have been recorded (Cubitt & Holzel, 1980). Since the initial observation of HRV particles in the duodenal mucosa of children suffering from acute gastroenteritis (Bishop *et al.* 1973) the epidemiology of HRV has been extensively investigated. In countries with temperate climates the majority of HRV infections occur in the winter months, whereas in tropical countries HRV infections are recorded throughout the year (Middleton, Szymanski & Petric, 1977; Soenarto *et al.* 1981).

Despite initial difficulties in preparing reagents, serological analysis of HRV has identified four serotypes, defined by a surface virion polypeptide, and two subgroups, defined by an internal virion polypeptide (Beards *et al.* 1980; Kalica *et al.* 1981*b*; Killen & Dimmock, 1982; Wyatt *et al.* 1983). The influence of serological variation on HRV epidemiology has yet to be established.

Table 1. *Source of faecal samples analysed.*

Year	City Hospital Aberdeen	Royal Aberdeen Childrens Hospital	Non-hospitalized children	Total
1982	55	6	4	65
1983	58	19	129	206
1984	87	15	335	437
Total	200	40	468	708

The genome of HRV is made up of 11 pieces of RNA which can be resolved by gel electrophoresis, and a number of workers have used differences in the electrophoretic mobilities of the genome RNA segments to distinguish HRV isolates collected during epidemiological investigations (reviewed by Estes, Graham & Dimitrov, 1984). Differences in the mobilities of RNA segments between HRV isolates define electrophoretotypes of the virus. Using this criteria, extensive variation among HRV isolates has been demonstrated with up to 32 distinct electrophoretotypes being identified during a 2-year period (Spencer, Avendano & Araya, 1983). Co-circulation of electrophoretotypes within the population during HRV epidemics has been reported although there appears to be a succession in the predominant electrophoretotype identified (Rodger *et al.* 1981). No correlations have been made between HRV electrophoretotypes and the severity of illness, although Rodger *et al.* (1981) identified particular electrophoretotypes as being associated with HRV infections of neonates. The relationship between HRV serotype and electrophoretotype is not clear but a correlation has been demonstrated between subgroups and the relative mobility of the two smallest RNA segments. Viruses with fast migrating RNA segments 10 and 11 ('long' RNA profiles) correspond to subgroup II while viruses with slow migrating segments 10 and 11 ('short' RNA profiles) belong to subgroup I (Kalica *et al.* 1981a).

This paper describes our results of investigating HRV epidemiology by RNA electrophoresis in north-east Scotland between 1982 and 1984.

#### MATERIALS AND METHODS

##### *Collection of faecal samples and detection of HRV*

Faecal samples were obtained from 708 patients suffering from diarrhoeal illness who were admitted to either the Royal Aberdeen Children's Hospital or the City Hospital, Aberdeen, between January 1982 and December 1984. Non-hospitalized patients who were treated by their general practitioner at home were also investigated in the later part of the survey. Table 1 shows the source of faecal samples tested between 1982 and 1984.

For the diagnosis of rotavirus infection, a 10% faecal suspension was prepared in phosphate buffered saline (PBS) and clarified at 3000 g for 30 min. It was then tested by one of three methods: (a) direct examination by negative staining and electron microscopy, thus identifying rotavirus according to the virion morphology; (b) agglutination of latex particules coated with rotavirus antiserum (Rotalex, Orion Diagnostica, Helsinki, Finland); (c) demonstration of virus antigen by a

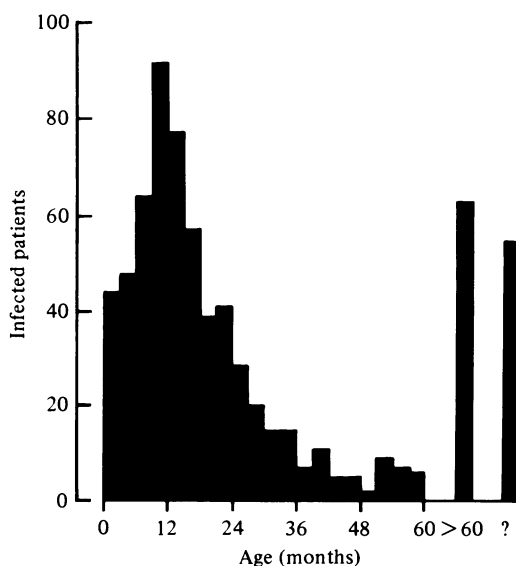


Fig. 1. Age distribution of infected patients between January 1982 and October 1984. (? , No age available).

commercially available enzyme linked immunosorbent assay (ELISA) (Rotazyme, Abbott Laboratories). The last two procedures were performed according to the manufacturer's instructions. A diagnosis by either electron microscopy or polyacrylamide gel electrophoresis (PAGE, see below), was considered absolute and no confirmatory test was performed. However, specimens positive by either ELISA or latex agglutination were then tested by PAGE and, in some cases, by at least one other method; results which were not confirmed were considered negative for the purpose of this study.

#### *Analysis of HRV RNA by polyacrylamide gel electrophoresis (PAGE)*

The preparation of HRV RNA from faecal samples and subsequent analysis by PAGE has been described previously (Cash, 1982) except that 0.4% SDS was present in the faecal suspension during the ultracentrifugation stage. To aid in the comparison of gels processed on different occasions, virion RNA prepared from a tissue culture-adapted isolate of bovine rotavirus (BRV), provided by Dr S. McNulty (Veterinary Research Laboratories, Stormont, Belfast), was used as a marker. The bovine rotavirus was grown in BS-C-1 cells as described previously (Cash, 1982) and virion RNA prepared as for HRV genome RNA from faecal samples.

## RESULTS

### *Characteristics of the infected population*

The geographical distribution of patients infected with HRV reflected the general population distribution for the Grampian region of north-east Scotland (data not shown). During 1982, children aged less than 5 years were examined for

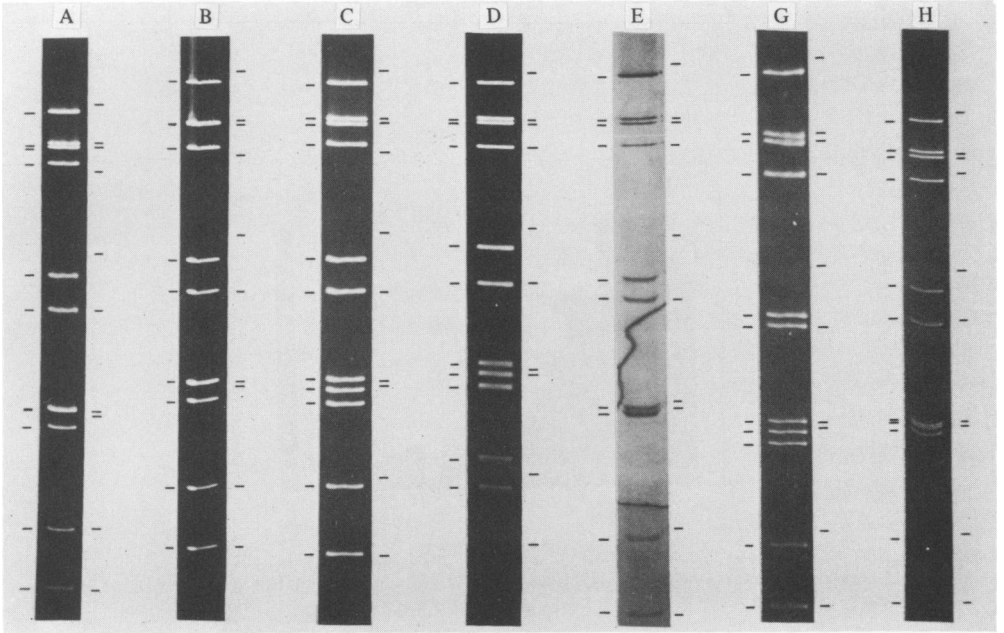


Fig. 2. HRV electrophoretotypes identified between January 1982 and September 1984. To the left of photograph of each gel track are indicated the locations of the HRV RNA segments and to the right of the photograph are shown the locations of the BRV RNA segments used as markers. This figure is a composite of a number of different gels. Electrophoretotypes F and Q had 'long' RNA profiles and type U a 'short' RNA profile (data not shown).

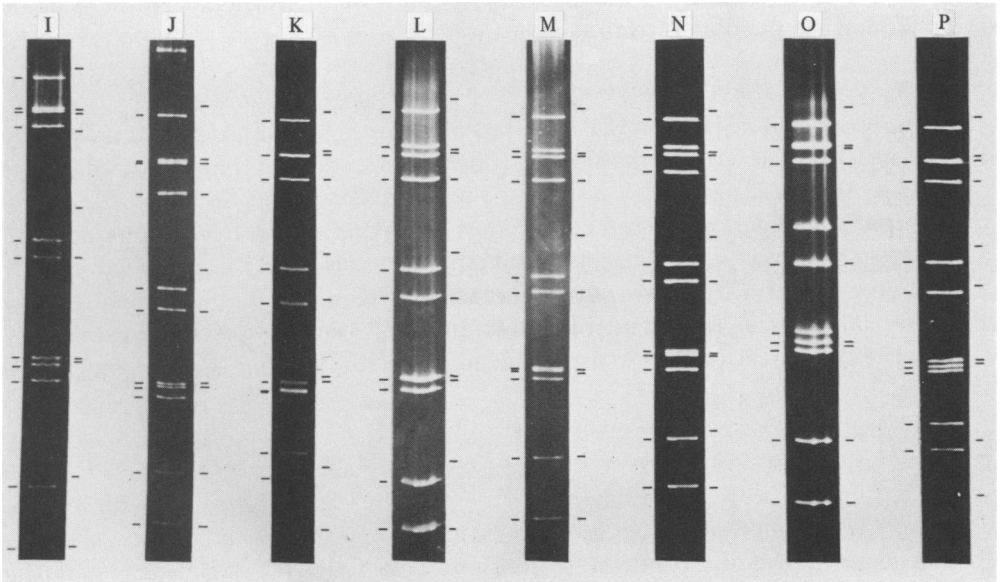


Fig. 3. HRV electrophoretotypes identified between January 1982 and September 1984. See legend to Fig. 2.

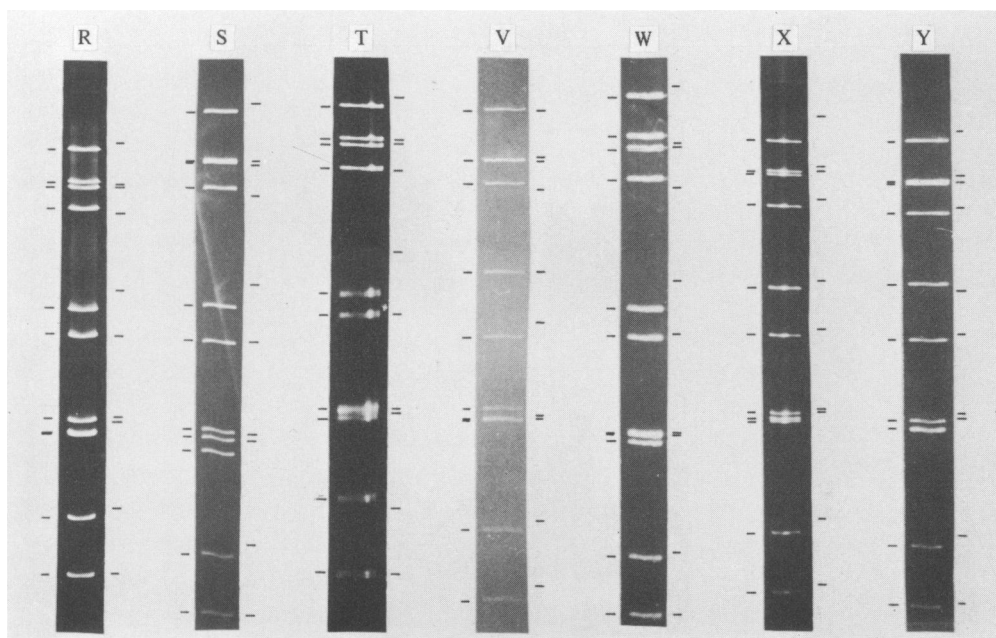


Fig. 4. HRV electrophoretotypes identified between January 1982 and September 1984.  
See legend to Fig. 2.

rotavirus whereas in 1983 and 1984 patients up to 12 years of age were routinely screened. The age distribution for infected patients identified between 1982 and 1984 is shown in Figure 1. Of the patients, 83% were aged under 5 years with a mean age of approximately 18 months. During the epidemic periods of 1983 and 1984, adults were also screened for HRV infection and positive findings were recorded in 31 patients over 20 years of age; 13 of these were older than 60 years. This must be considered an underestimate for adult infections because adults were not routinely screened for HRV infection. Overall, there was an excess of male compared to female patients (381 males and 302 females; data were not available for 25 patients). This was significantly different from an even sex distribution ( $P < 0.01$ ). The reason for the excess of males compared to females was unknown although a similar distribution was reported by Brandt *et al.* (1979). There was no significant difference in age distribution of the male and female patients.

#### *Identification of HRV electrophoretotypes*

Between January 1982 and December 1984, 708 patients were diagnosed as having a rotavirus infection. Virus RNA was extracted from 840 faecal specimens containing rotavirus; 700 (83%) yielded RNA suitable for analysis. This total also includes multiple specimens from individual patients. Twenty-five different HRV electrophoretotypes were identified (Figs. 2-4) and were assigned a letter on an arbitrary basis for identification. The grouping of HRV isolates into electrophoretotype groups was based upon comparison with the BRV marker RNA and, if possible, verification by repeat analyses of the same stool sample or, when sufficient material was available by co-electrophoresis of RNA samples. For 24 patients,

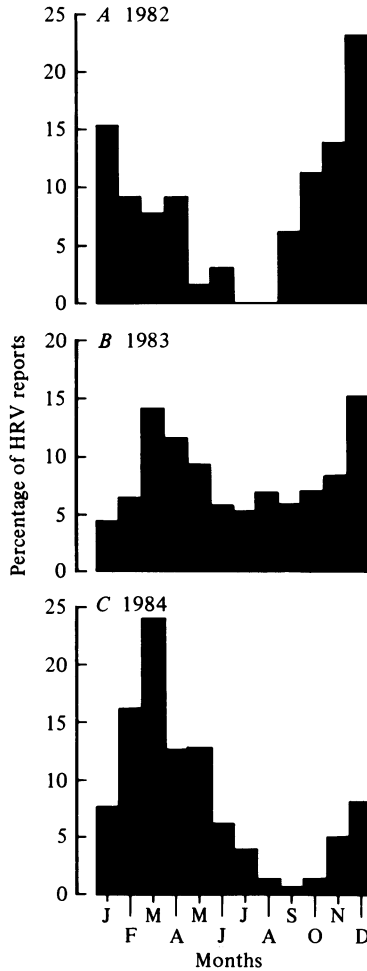


Fig. 5. Distribution of the reports of HRV infections during 1982(A), 1983(B) and 1984(C).

serial faecal specimens up to 7 days apart gave identical RNA profiles (data not shown). The 25 electrophoretotypes could be classed as either 'long' or 'short' RNA types according to the relative migration of RNAs 10 and 11. On this basis, the 'long' RNA types showed the greater degree of variation (21 electrophoretotypes) compared to the 'short' RNA types which were represented by only 4 types.

There was no correlation of any particular type with either hospitalized or non-hospitalized patients. The major virus electrophoretotype present at any one time during the virus epidemic was the same for each group. However, non-hospitalized patients yielded the greater range of electrophoretotypes which included the majority of minor virus types. This was probably due to the larger number of specimens obtained from this group. In the following account we have considered data from the two patient groups together. There was no evidence for any association between particular HRV electrophoretotype and the age or sex of the patient. Electrophoretotypes C, L, N and R were isolated from 6 of the 13 patients over 60 years of age.

Table 2. *Distribution of electrophoretotypes during 1982*

Virus type*	Month (1982)											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
A	8	1	3	2	—	—	—	—	—	—	—	—
B	1	1	1	4	1	—	—	—	2	—	—	—
C	—	1	—	—	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	2	5	8	14
Long†	9	3	4	6	1	—	—	—	2	—	—	—
Short†	—	—	—	—	—	—	—	—	2	5	8	14

\* See Figs. 2-4.

† Classified according to the relative migration of RNA segments 10 and 11.

Table 3. *Distribution of electrophoretotypes during 1983*

Virus type*	Month (1983)											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
A	—	2	5	9	11	8	5	4	2	2	—	3
B	2	2	5	2	1	—	—	—	—	2	1	4
C	—	—	—	—	1	—	—	—	—	—	—	1
D	5	6	5	1	1	—	1	—	—	—	—	—
E	—	1	—	—	—	—	—	—	—	—	1	1
G	—	—	1	—	—	—	—	—	—	—	—	—
H	—	—	1	2	—	—	—	—	1	—	—	—
I	—	—	—	1	—	—	—	—	—	—	—	1
J	—	—	—	—	1	—	—	—	—	—	—	—
K	—	—	—	—	—	—	—	2	—	—	—	—
L	—	—	—	—	1	—	—	—	1	2	—	2
M	—	—	—	—	—	—	—	—	—	—	4	—
N	—	—	—	—	—	—	—	1	—	1	3	4
O	—	—	—	—	—	—	—	—	—	1	—	—
P	—	—	—	—	—	—	—	—	—	1	4	6
R	—	—	—	—	—	—	—	—	—	—	—	2
T	—	—	—	—	—	—	—	—	—	—	—	1
Long†	2	5	12	14	15	8	5	5	4	8	9	20
Short†	5	6	5	1	1	—	1	2	—	1	4	6

\* See Figs. 2-4.

† Classified according to the relative migration of RNA segments 10 and 11.

*Distribution of rotavirus electrophoretotypes with time*

The temporal distribution of all reports of HRV infections between 1982 and 1984 is shown in Fig. 5. In 1982, HRV infections showed the typical seasonal pattern with a higher incidence in the number of reports during the winter months (January to March and September to December) compared to the summer months (April to August). A similar trend was found for 1984. In contrast, during 1983 a greater proportion of rotavirus infections was reported between April and August (39% of reports for 1983 compared to 13% of reports for 1982 for the same period). This was not simply due to the larger number of samples tested in 1983 because during these months 27% of specimens tested from hospitalized patients were positive for rotavirus compared to 6.5% for 1982.

Table 4. *Distribution of electrophoretotypes during 1984*

Virus type*	Month (1984)											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
A	—	6	—	3	5	1	4	—	1	—	2	2
B	2	3	10	9	3	1	—	1	—	—	—	—
C	1	28	34	7	3	5	4	—	1	—	—	—
D	—	—	—	—	2	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	—	2	—	—
G	—	—	—	1	—	—	—	—	—	—	—	1
J	—	1	—	1	—	—	—	—	—	—	—	—
K	—	—	—	—	—	2	—	—	—	—	—	—
L	1	—	—	—	—	—	—	—	—	—	4	6
N	7	5	9	4	5	1	3	—	—	—	—	—
O	1	1	—	—	—	—	—	—	—	—	—	—
P	5	2	2	—	—	—	—	—	—	—	—	—
Q	—	—	—	—	—	—	—	—	—	—	1	—
R	5	8	30	31	7	3	2	—	—	—	—	—
S	—	—	1	—	1	—	—	—	—	—	—	1
U	—	—	—	—	—	—	—	—	—	—	1	—
V	1	—	—	—	—	—	—	—	—	—	—	—
W	—	1	—	—	—	—	—	—	—	—	—	—
X	—	—	—	—	—	—	—	1	—	—	—	—
Y	—	—	—	—	—	—	—	—	1	—	—	—
N.T. ‡	—	—	4	—	23	16	17	5	—	1	9	25
Long †	17	54	84	56	24	11	13	2	3	2	7	10
Short †	5	2	2	—	—	2	—	—	—	—	1	—

\* See Figs. 2-4.

‡ Not tested for virus electrophoretotype.

† Classified according to the relative migration of RNA segments 10 and 11.

All rotavirus-positive faecal specimens received during 1982 and 1983 were processed to determine the virus electrophoretotype. During 1984, only selected specimens were processed for typing. These data are shown in Tables 2, 3 and 4. In 1982, four HRV variants were identified. Three (types A, B and C) had 'long' RNA profiles and were confined to the early months of 1982 (January to April). Type C was identified in only 1 out of 23 typed faecal specimens at this time. Two variants (A and B) co-circulated during this period with type A being in excess. From September to December, there was a major shift in the predominant electrophoretotype to a 'short' RNA profile (type D). During this period only 2 out of 31 typed viruses had 'long' profiles, both of type B. The type D was also evident during the early part of 1983 (up to June). In a preliminary investigation of HRV types circulating in 1981, the RNA from 30 out of 72 HRV positive faecal specimens was typed; none of these showed 'short' RNA profiles (unpublished data).

The distribution of the electrophoretotypes in 1983 and 1984 was more complex than in 1982 with 16 HRV variants in 1983 and 20 variants in 1984. The larger number of electrophoretotypes identified was probably due to the increased number of viruses typed in these 2 years compared to 1982. Despite this, however, the majority of the viruses typed could be classified into a relatively small number of



electrophoretype classes. In 1983, viruses classed into four types (A, B, D and P) comprised 73% of the total number of specimens typed. Similarly, for 1984 86% of the typed specimens were classed into five main electrophoretotypes (A, B, C, N and R). The remaining 15 electrophoretotypes identified during 1984 were represented by much smaller groups of specimens. Unlike 1982, there was extensive co-circulation of viruses with 'short' and 'long' RNA profiles with the latter in excess - 78% of specimens in 1983 and 95% in 1984. There was evidence of simultaneous circulation of several HRV electrophoretotypes with up to nine distinct variants being detected during a single month (December 1983).

#### DISCUSSION

Despite the difficulties in interpretation, electrophoretic typing of HRV genome RNA has been useful in studying virus epidemiology. Our results agree with those of other workers (Rodger *et al.* 1981; Spencer, Avendando & Araya, 1983; Follett *et al.* 1984) in demonstrating extensive variation at the molecular level between HRV isolates. In our investigations, we assumed that viruses in which all detectable RNA segments co-migrated were in the same electrophoretype group. While this may be valid when viruses are closely related (e.g. duplicate samples from one patient or from localized HRV outbreaks) it may not be valid for widely separated virus isolates. Evidence for the latter comes from the work of Clarke & McCrae (1982) who demonstrated that co-migrating RNA segments from different BRV isolates had distinct nucleotide sequences. Consequently, the electrophoretotypes identified by PAGE may in fact represent the minimum number of circulating virus variants. The function of the extensive variations observed for HRV is still unclear. No correlation was reported by Beards (1982) between the genome RNA profile and virus serotype. The possibility that the changes in the virus genome reflect alterations in virus pathogenicity and transmissibility has still to be fully investigated.

Between 1982 and 1984 the following general pattern for HRV epidemiology emerged. During a single epidemic period a small number of electrophoretotypes circulated in the population and made up the majority of viruses identified. Superimposed on this was a larger number of minor electrophoretotypes co-circulating sporadically with no clearly defined pattern. These latter virus types may represent strains introduced into the population which fail to become established, possibly due to either an antigenic characteristic of the virus or another biological property such as low transmissibility between susceptible persons. An example of this may be electrophoretype M which showed limited spread within a single locality even though all the affected patients were admitted to hospital in Aberdeen. In contrast, evidence was obtained on the spread of type B, the third most common variant found, when an infected child was admitted to hospital and two children in contact with this patient developed an infection with an HRV of type B (unpublished data). Two electrophoretotypes (A and B) persisted in the population throughout the 3-year investigation although their prevalence varied in separate epidemics. Recently, Konno *et al.* (1984) reported that during HRV epidemics in Japan a dominant electrophoretype was evident in the early months of the outbreak with more extensive co-circulation of differing virus electrophoretotypes in later months.

From our own data, a small number of dominant electrophoretotypes was observed throughout an epidemic with no major change in their distribution. At all stages, minor variants were co-circulating. The difference between these two observations may be due to the high incidence of HRV during the summer of 1983, one of the inter-epidemic periods of our study, which blurred the start of the HRV epidemic over winter 1983/1984. However, a dominant electrophoretotype was found at the start of the winter of 1982/1983 when it was the only variant observed for 3 months. It was of interest that this electrophoretotype corresponded to a serological subgroup not previously recognized in this area and this may have accounted for its dominance. Rodger *et al.* (1981) postulated a cyclical change in HRV types over a long period of time. None was apparent over the 3 years of our investigation.

We do not understand how new HRV variants are generated. Spencer *et al.* (1983) identified viruses with more RNA segments than the usual complement of 11 in 10% of samples out of 142 typed faecal specimens. These workers also demonstrated a gradual change in the RNA profile of viruses in serial faecal specimens from three infants suggesting either a sequential or simultaneous infection with distinct HRV variants. These data would support both the gradual 'drift' of one virus variety into another and the reassortment of viruses during a dual infection of a patient, so generating new electrophoretotypes. Garbarg-Chenon, Bricout & Nicolas (1984) have recently provided experimental evidence that human rotaviruses can produce reassortant viruses under *in vitro* conditions. In our study only 1 out of 700 typed viruses showed an RNA profile with additional RNA segments; RNAs 2 and 6 appearing as double bands (unpublished data). In addition, multiple samples from 24 patients showed no changes in the electrophoretic mobilities of the virus RNAs during infection. It is possible that dual infections may not be a common phenomenon and may be influenced by the immune status of the patient in combination with the types of virus circulating in the population. However, mutations may have occurred which did not affect the electrophoretic mobilities of the RNA segments.

The identification of the extensive molecular variation among HRV isolates is important in understanding the spread of this significant human pathogen. The relationships between the molecular variation, changes in the serological characteristics and virus pathogenicity have still to be fully elucidated.

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