Paediatric gastroenteritis in the eastern Malaysian state of Sarawak: an epidemiological and clinical study

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Abstract
Over a period of 2 months, 35 of 69 (51%) cases of juvenile diarrhoea studied in eastern Malaysia were associated with rotavirus excretion; rotavirus-associated diarrhoea occurred most commonly in the 6–24 month age group. Polyacrylamide gel electrophoresis (PAGE) of genome ribonucleic acid showed that only 4 rotavirus electropherotypes could be detected. Of those, 2 predominated and 2 were detected only once each; one of these may have been a reassortment of the two predominant electropherotypes. Analysis of the clinical features of patients excreting rotavirus subgroup 1 or 2, determined by PAGE, demonstrated that rotavirus subgroup 1 was associated with more hypotonic dehydration and need for intravenous therapy; lethargy was significantly more common among those excreting rotavirus subgroup 2.

Introduction
Diarrhoeal diseases constitute an important and complex health problem among children in Malaysia, causing high morbidity and mortality with consequent retardation in growth and development. However, although the importance of viruses, especially rotaviruses, in the aetiology of paediatric diarrhoeal diseases is now well established, little has been reported on the occurrence of such viruses in East Malaysia.

The field part of the study was undertaken by two medical students (R.A.B. and P.H.M.) on a two-month elective project. They examined the role of viruses in, and the clinical features of, diarrhoea among children admitted to two hospitals in Sarawak during January and February 1986. The genome of the rotaviruses detected was examined at St Thomas’s Hospital by polyacrylamide gel electrophoresis (PAGE) to determine the extent of rotavirus variation.

Materials and Methods
Patients
The main study group consisted of children under 5 years of age, admitted with a diagnosis of acute gastroenteritis to Lau King Howe Hospital, Sibu, Sarawak, between 7 January and 22 February 1986. On admission the patients were examined and a stool sample collected. A questionnaire was completed on discharge. Of 78 such patients admitted during the period of study, 69 (89%) were enrolled. The period studied covered the transition from the wet to the dry seasons (late January).

Faecal samples and clinical details were also collected from a small control population at Sibu (10 patients under 5 years of age admitted with a diagnosis other than acute gastroenteritis), and from 10 patients with acute gastroenteritis admitted to a small rural hospital in Kapit, about 115 km from Sibu.

These patients came from a predominantly rural environment with poor sanitation. Patients admitted to the Lau King Howe Hospital, Sibu, were from a comparable rural setting as well as from an urban environment with relatively good sanitation and housing, but with some overcrowding.

Dehydration was assessed clinically in terms of skin turgor, the state of the mucous membranes and fontanelles, and the intracranial pressure (INSLEY & WOOD, 1982). The tonicity of dehydration was determined by the sodium ion concentration: if it was less than 134 mmol per litre the dehydration was considered to be hypotonic; if greater than 147 mmol per litre then hypertonic dehydration was diagnosed.

Virus detection
All faecal samples were examined in Sibu by enzyme-linked immunosorbent assay (ELISA) for rotavirus antigen using the Dakopatts Rotavirus ELISA Kit (Dako, Copenhagen, Denmark). An aliquot of each sample was frozen at −20°C and transported to St Thomas’s Hospital, London, for examination, after negative staining, by electron microscopy (EM) and PAGE (GRILLNER et al., 1985). Electropherotypes were designated 1 and 2, representing the ‘short’ and ‘long’ electropherotypes respectively (ROGER et al., 1981). Different electropherotypes within each of these subgroups were labelled alphabetically.

Results
Of 69 patients admitted to Lau King Howe Hospital, rotavirus was detected by EM, ELISA, or PAGE in 35 (51%). Adenovirus was detected in 2 patients and coronavirus-like particles in one (Table 1). Patients with rotavirus-positive diarrhoea were significantly older than those in whom rotavirus could not be detected (P<0.001), the major age group affected being from 6 to 24 months of age (Table 2). Three of 10 (30%) controls in Sibu were excreting rotaviruses, as were 3 of 10 patients at Kapit. One patient at Kapit was also excreting astroviruses. Throughout the period studied no patients died of gastroenteritis.

There were no significant differences between rotavirus and non-rotavirus associated diarrhoea in terms of clinical features, other than the observation that patients excreting rotaviruses were more likely to present earlier, most within 4 d (P<0.05) (Table 3).

Comparison of ELISA, EM, and PAGE for the detection of rotaviruses in faecal samples indicated that the 3 techniques were of similar sensitivity.
Table 1. Virus detection in faecal samples from paediatric patients and controls

|               | Total | Rotavirus | Adenovirus | Astrovirus | Coronavirus |
|---------------|-------|-----------|------------|------------|-------------|
| Sibu patients | 69    | 35 (51%)  | 2          | -          | 1           |
| Sibu controls | 10    | 3 (30%)   | -          | -          | -           |
| Kapit patients| 10    | 3 (30%)   | -          | 1          | -           |

Table 2. Age distribution of rotavirus-associated diarrhoea in Sibu

| Age range (months) | Rotavirus positive | Rotavirus negative |
|--------------------|--------------------|-------------------|
| 0–6                | 9 (33%)            | 21                |
| 7–24               | 21 (67%)           | 9                 |
| 25–60              | 5 (55%)            | 4                 |

Table 3. Clinical features of rotavirus positive and rotavirus negative patients

| Clinical feature                | Rotavirus positive (N=35) | Rotavirus negative (N=34) |
|--------------------------------|---------------------------|---------------------------|
| Sex: Male                      | 21                        | 25                        |
| Female                         | 14                        | 9                         |
| Vomiting                       | 27                        | 22                        |
| Fever                          | 19                        | 12                        |
| Dehydration: <6%               | 27                        | 28                        |
| ≥6%                            | 8                         | 6                         |
| Tonicity of dehydration       |                           |                           |
| Isotonic                       | 21                        | 23                        |
| Hypotonic                      | 9                         | 8                         |
| Irritability                   | 8                         | 8                         |
| Lethargy                       | 9                         | 6                         |
| Pharyngeal erythema            | 13                        | 10                        |
| Tonsillar exudate              | 2                         | 0                         |
| Rhinitis                       | 0                         | 0                         |
| Red tympanic membrane          |                           |                           |
| with loss of landmarks         | 1                         | 1                         |
| Ronchi/whistling               | 1                         | 1                         |
| Palpable cervical lymph nodes  | 0                         | 1                         |
| No. of stools per day: <6      | 22                        | 23                        |
| ≥6                             | 13                        | 11                        |
| Blood in stool                 | 3                         | 1                         |
| Mucus in stool                 | 4                         | 2                         |
| Days of illness before admission: 0–3 | 30                    | 20                        |
|                                    | 4–7                       | 5                         | 10                        |
|                                    | ≥8                        | 0                         | 4                         |
| Days in hospital: 0–3           | 1                         | 4                         |
|                                    | 4–7                       | 25                        | 15                        |
|                                    | ≥8                        | 9                         | 8                         |
| Unknown                         | 0                         | 7                         |
| Duration of intravenous therapy (d): none | 4                     | 9                         |
|                                    | 1–3                       | 13                        | 11                        |
|                                    | 4–7                       | 16                        | 6                         |
|                                    | ≥8                        | 2                         | 8                         |
| Unknown                         | 0                         | 4                         |
| Duration of diarrhoea (d): 1–3   | 3                         | 2                         |
|                                    | 4–7                       | 23                        | 14                        |
|                                    | ≥8                        | 9                         | 14                        |
| Use of drugs/antibiotics        | 14                        | 12                        |
| Race: Chinese                   | 12                        | 8                         |
| Iban/Sea Dayak                  | 21                        | 21                        |
| Other                           | 2                         | 5                         |
| Type of feeding: Formula        | 21                        | 28                        |
|                                | Breast                    | 4                         | 1                         |
|                                | Solid food                | 5                         | 4                         |
|                                | Mixed                     | 5                         | 1                         |

Figure. Electropherotypes (EPT) of rotaviruses identified from faeces of children in Sarawak.
A. EPT 1B (lane 1) is probably a reassortment of EPTs 1A (lane 2) and 2A (lane 3).
B. EPT 2B (lane 3) is not a reassortment of 1A (lane 2) and 2A (lane 1). Lanes 4 and 5 show double infections with EPTs 1A and 2A.

Table 4. Distribution of rotavirus electropherotypes in Sibu and Kapit

| Rotavirus electropherotype | Sibu patients | Sibu controls | Kapit patients |
|---------------------------|---------------|---------------|----------------|
| 1A                        | 24            | 24            | 1              |
| 1B                        | 1             | 0             | 0              |
| 2A                        | 7             | 1             | 2              |
| 2B                        | 1             | 0             | 0              |
| Negative                  | 3             | 1             | 0              |
| Total                     | 35            | 32            | 3              |

*a*Mixture of 1A and 2A.
"Patients designated 'unknown' were still in hospital when the study finished.

Rotavirus ribonucleic acid was detected in 37 faecal samples by PAGE. Four different electropherotypes could be identified (Figure). Two of these, designated 1B and 2B, were detected only once each. Of the others, 27 (72%) exhibited a 'short' electropherotype designated 1A, and 7 (10%) a 'long' electropherotype, 2A. Two of these samples contained a mixture of 1A and 2A (Table 4, Figure B). Patients excreting the short electropherotype 1A demonstrated significantly more hypotonic dehydration.
Table 5. Significant clinical differences between patients excreting rotavirus subgroup 1 or subgroup 2

| Clinical feature                        | Rotavirus subgroup 1 (n=25) | Rotavirus subgroup 2 (n=8) |
|----------------------------------------|-----------------------------|---------------------------|
| Tonicity of dehydration                |                             |                           |
| Isotonic                               | 16                          | 7                         |
| Hypotonic                              | 5                           | 0                         |
| Lethargy                               | 4                           | 4*                        |
| Duration of intravenous therapy        |                             |                           |
| None                                    | 2                           | 3                         |
| 1-3 d                                   | 7                           | 5                         |
| 4-7 d                                   | 14                          | 0                         |
| <7 d                                    | 2                           | 0*                        |

P<0.001  bP<0.05

Discussion

This study demonstrated that rotaviruses were associated with over 50% of cases of acute diarrhoea among children in the central region of Sarawak. However, the detection of rotaviruses in 3 of 10 children not suffering from diarrhoea implied that this association was not unequivocal. High levels of asymptomatic rotavirus infection among children have been reported previously (CHAMPSAUR et al., 1984) and asymptomatic infection is known to occur among the newborn, and in older children experiencing reinfection. That the major age group affected was 6 to 24 months of age is in agreement with other studies (e.g., KAPKIAN et al., 1976; MIDDLETON et al., 1977).

The initial assays for rotavirus used the Dakopatts ELISA kit and were performed in Sarawak on freshly collected samples. This test, the reagents of which were carried in hand luggage from London, provided the local clinicians with a cheap, simple test, the accuracy of which was confirmed by subsequent analysis by EM and PAGE. Although the local clinicians welcomed the provision of a rotavirus diagnostic service the results did not affect patient management, in that patients with rotavirus-induced diarrhoea as well as other forms responded equally favourably to oral rehydration if treatment was given early. Thus the service was not continued after completion of the study.

Many studies have shown that, in contrast to diarrhoea caused by other micro-organisms, rotavirus-induced diarrhoea is characterized by increased vomiting, dehydration, and diarrhoea (e.g., RODRIGUEZ et al., 1977; HIEBER et al., 1978). Some investigators have also noted an increase in upper respiratory tract symptoms (HIEBER et al., 1978; LEWIS et al., 1979). However, in this study no difference could be seen between rotavirus-associated and non-rotavirus-associated diarrhoeas other than the observation that rotavirus-associated cases presented sooner after onset than others, presumably reflecting the abrupt onset of the illness or the relatively short duration of virus excretion. Respiratory symptoms were almost totally absent.

Only 4 rotavirus electropherotypes were detected, and of these 2, designated 1B and 2B, were found only once each. Of the 32 rotavirus positive samples from patients at Sibu which were also PAGE positive, 24 (75%) showed the same short electropherotype 1A, and 7 (22%) the same long electropherotype 2A. One sample contained both electropherotypes. Of the rotavirus positive samples from the control group at Sibu and the patients at Kapit, 3 contained variant 1A, 2 had variant 2A, and one had a mixture of both. Simultaneous infection with more than one rotavirus electropherotype has been reported previously (LOURENCO et al., 1981; SVENSSON et al., 1986), and it has been proposed that such infections may generate reassortment viruses (FLORES et al., 1985). As all bands in 1B co-ran with bands in either 1A or 2A (Figure A), it seems likely that electropherotype 1B was a reassortment of 1A and 2A, although confirmation of this hypothesis would require the use of techniques such as oligonucleotide mapping. Electropherotype 2B cannot have been generated from a mixed 1A and 2A infection; whether the patient involved was of local origin could not be established.

Studies on the extent of electropherotypic variation of rotaviruses in large populations often demonstrate that many variants co-exist (CROXON & BELLAMY, 1979; RODGER et al., 1981). Studies in small populations, in which single variants are observed, suggest that there is a minimum population size which can maintain the virus as an endemic pathogen (ALBERT et al., 1983). Such may be the case in the population studied here, as only two electropherotypes predominated. The same electropherotypes (1A and 2A) predominated in locations about 115 km apart, whereas in a similar 15 month study in Kenya only one of 34 electropherotypes detected in locations 480 km apart was found in both areas (CHIBA et al., 1984). Population movement between Sibu and Kapit is quite extensive, and the similarity of electropherotypes probably resulted from the spread of virus to a population too small to maintain endemic rotavirus infection. Although CHIBA et al. (1984) reported frequent exchange of people between Nairobi and Mombasa, both areas are densely populated and rotavirus infection is endemic all year round.

Subgroup 1 rotaviruses exhibit a slower running segment 10 and 11 duplet, a short pattern, whereas subgroup 2 rotaviruses show a longer pattern (RODGER et al., 1981; DYALL-SMITH & HOLMES, 1981). Although one study has recently reported a subgroup 1 rotavirus with a long electropherotype (NAKAGOMI et al., 1985), we have assumed that the electropherotypic analysis can be used to give a presumptive diagnosis of subgroup specificity, and have analysed the clinical features of the two groups.

The one similar study had shown no differences in severity of fever, vomiting or diarrhoea between the two subgroups, although the syndrome was thought to be of longer duration with subgroup 2 rotaviruses, which the authors suggested might indicate that subgroup 2 rotaviruses were more virulent (WHITE et al., 1984). In most studies of diarrhoea subgroup 2 rotaviruses predominate, again suggesting that these viruses are more virulent (YOLKEN et al., 1978; WHITE et al., 1984), although rotavirus subgroup 1 outbreaks have also been reported (ALBERT et al., 1983). In this study we observed that diarrhoeal
illness associated with the short electropherotype 1A, the predominant variant, and presumed subgroup 1, involved more hypotonic dehydration and necessity for intravenous therapy than that associated with the long electropherotype 2A, presumed subgroup 2. Lethargy was significantly more common with the long electropherotype 2A. Analysis of all relevant investigations suggests, therefore, that subgroup specificity and severity of disease are unlikely to be linked. A recent report linking rotavirus virulence in newborn mice with gene segment 4 (OFFIT et al., 1986) seems to confirm this conclusion.

Worldwide efforts are currently being made to reduce the levels and severity of diarrhoeal disease among children. Surveys such as this will not only provide data on which future vaccination programmes can be designed but will also be of value in assessing the success of such interventions.

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