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Rotavirus serotypes causing acute diarrhoea in hospitalized children in Yogyakarta, Indonesia during 1978–1979

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Summary. Rotavirus strains in stool specimens from 111 children aged 3–24 months admitted to hospital in Yogyakarta, Indonesia for treatment of acute diarrhoea were serotyped using VP_7 serotype specific monoclonal antibodies in a double sandwich enzyme immunoassay. A serotype could be assigned to 59 of 111 specimens (53%). Inability to assign a serotype to 47% of specimens was probably due to loss of the outer capsid during transport of specimens from Indonesia to Australia. All four major human rotavirus serotypes were detected during the 15 month survey from June 1978 to August 1979, including one serotype 1, 5 serotype 2, 31 serotype 3, and 21 serotype 4 strains. One additional strain reacted with serotype 3 and 4 Mabs. Serotype 3 strains showed intratypic variation.

The relative frequency of serotypes 2, 3, and 4 varied during the 15 months and appeared to be influenced by climatic changes associated with dry and wet seasons. Vaccine strategies must take account of comparatively rapid changes of predominant serotypes in a community and are only likely to be successful if comprehensive immunity can be established simultaneously against the four major human serotypes.

Introduction

Rotavirus infection is the single most important cause of severe acute diarrhoea requiring admission to hospital of young children throughout the world. Development of an effective vaccine has become a priority of the World Health Organization in an attempt to reduce mortality in young children in developing countries [3]. Field trials of candidate rotavirus vaccines in developing countries have yielded variable results to date. Candidate vaccines derived from animal rotavirus strains have shown high efficacy, when tested in a field setting where

strains causing severe infection have been predominantly of the same serotype as that of the candidate vaccine [9]. The same vaccines have been ineffective when the predominant field strains have differed in serotype from the candidate strains [4].

In order to develop effective vaccine strategies it is necessary to compile information about the geographical and temporal distribution of rotavirus serotypes commonly isolated from infants with diarrhoea. Limited surveys of rotavirus serotypes (using assays incorporating absorbed hyperimmune sera) have shown a worldwide distribution of serotypes 1, 2, 3, and 4 [18]. These assays detect two outer capsid proteins (VP_4, VP_7), and recent evidence indicates that the serotype assignments correpond mainly with epitopes on the outer capsid glycoprotein VP_7 [13]. The development of serotyping assays incorporating monoclonal antibodies reacting with epitopes on VP_7 of human rotaviruses [8, 16] now permits serotyping of larger numbers of strains of human rotaviruses derived from field surveys.

We previously published results of a 13 month survey of the importance of rotavirus infection in aetiology of acute diarrhoea in children aged 0–24 months admitted to one hospital in Yogyakarta, Indonesia during 1978–1979 with acute diarrhoea [15]. The same specimens were subsequently analysed by electrophoresis of genome RNA and shown to comprise nine different electropherotypes [1]. This study now reports the relative frequency of human rotavirus serotypes in specimens collected during that survey.

Materials and methods

Specimens

Stool specimens from 111 children (62 males) aged 3–24 months known to be positive for rotavirus were studied. The children had been admitted to paediatric wards of Gadjah Mada Hospital, Yogyakarta, Indonesia for treatment of acute diarrhoea between June 1978 and August 1979. Specimens were obtained within 24 hours of admission to hospital from 35 children of medium-high socioeconomic level and 76 children of low socioeconomic level (defined as from rural and urban families with an income less than US$15.00 per month and unable to pay for hospital treatment). Specimens were initially stored at − 20 °C in Yogyakarta and transported by air in batches to Melbourne, Australia every 3–4 months. Electron miscroscopy had detected rotavirus particles as sole enteric pathogen in 107 children [15]. LT positive enterotoxigenic *Escherichia coli* had been identified in addition to rotaviruses in four children. Electrophoresis of genome RNA extracted from faecal specimens had allowed an electropherotype to be identified from 53 of the 111 specimens [1]. Rotavirus positive specimens used for serotyping had been stored at − 70 °C for 8–9 years as ultracentrifuged pellets prepared for electron microscopy.

Monoclonal antibodies

The derivation and classification of the neutralizing monoclonal antibodies used in the assay have been described previously [6, 7]. The serotype specificities have been confirmed using serum neutralization assays or solid-phase immune electronmicroscopy [11]. Six monoclonal antibodies that recognize VP_7 of Group A rotaviruses were used. Mabs RV 4 : 1, RV 4 : 2, RV 4 : 3 recognize epitopes on VP_7 of serotype 1 and permit classification into

monotypes 1a, 1b, 1c, [5]. Mab RV4:3 cross-reacts with serotype 3 viruses [7]. Mabs RV5:3, RV3:1, ST3:1 are specific for VP_7 of serotypes 2, 3, and 4 respectively. Mab RV-A recognizes an inner capsid epitope on VP_6 that is common to all Group A rotaviruses and was incorporated as a control to estimate amount of rotavirus antigen in each sample.

Rotavirus serotyping

Serotyping was performed using a double-sandwich serotyping enzyme immunoassay (EIA) as previously described [8]. Briefly, wells of polystyrene microtitre trays were separately coated with preimmune or hyperimmune rabbit antisera to human rotavirus serotypes 1–4 diluted optimally in phosphate buffered saline pH 7.2. After adsorption and washing, faecal extracts or rotavirus-infected or uninfected MA 104 cell harvests were incubated in wells, washed three times and serotype-specific monoclonal antibodies reactive with the same virus serotype as the hyperimmune coating sera were added [17]. Mab binding was detected with goat anti-mouse immunoglobulins conjugated to horseradish peroxidase (Silenus laboratories, Victoria, Australia) followed by substrate containing 3, 3^1, 5, 5^1 tetramethylbenzidine (Sigma) and stopping the reaction after 10 minutes using $2 M H_2SO_4$. A faecal sample was considered positive for a particular serotype if the OD 450 with sera to that serotype (P) was at least 2.0 times the mean OD 450 value of that sample reacted with sera in the non-reactive wells (N), i.e., P/N value >2.0.

Results

As judged by reaction with the group A Mab (RV-A) 110 of 111 rotavirus positive (EM) specimens contained sufficient particles to enable serotyping. No differences were observed in relation to socioeconomic level of the patients so the results from the two groups have been combined. A serotype could be assigned to 59/111 specimens (53%) including one serotype 1, 5 serotype 2, 31 serotype 3, and 21 serotype 4 strains. One specimen detected in August 1979 showed a dual reaction with serotype 3 and 4 monoclonals. Seven of the serotype 3 strains (denoting reaction with RV3:1 which is specific for an epitope on VP_7 of serotype 3) showed an additional positive reaction with RV4:3 which reacts with an epitope on VP_7 of serotype 1. These doubly reacting strains are designated serotype 3×1.

Figure 1 shows the frequency of detection of serotypes 1, 2, 3, and 4 in relation to climatic changes. Ambient temperatures and relative humidity vary little in Yogyakarta throughout the year, but seasonal changes in rainfall occur. A serotype could be assigned to 23 of 51 specimens (45%) obtained from patients admitted during the dry period of June to November 1978, to 28 of 44 specimens (65%) obtained during the wet period from December 1978 to April 1979, and to 8 of 16 specimens (50%) from the second dry period from May to August 1979. The change in climatic conditions was associated with a change in the dominant serotype. The majority (74%) of specimens serotyped during the first "dry" season were serotype 3 strains (including 7 serotype 3×1 strains). Serotype 3 strains persisted during the following wet and dry seasons but were identified in only 37% and 38% of typed specimens respectively. No serotype 3×1 strains were identified after November 1978. Serotype 4 strains increased in frequency

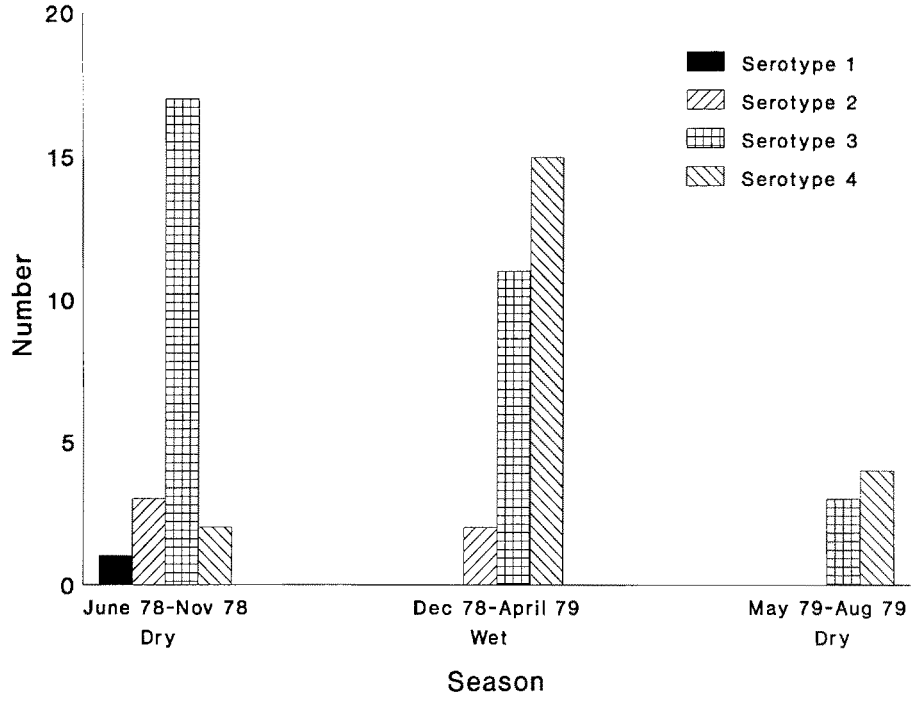

Fig. 1. Occurrence of human rotavirus serotypes in children admitted to hospital in Yogyakarta (Indonesia) in relation to climatic changes

from 9% to 54% during the wet season and persisted at this level of frequency during the following dry months.

Table 1 shows the age distribution of the children excreting known serotypes. The mean and median ages of children infected with serotype 3 (11.7 months, 11 months respectively) were higher than for those infected with serotype 4 (10.2 months, 9 months respectively). This difference reflects the fact that proportionately more of the children infected with serotype 3 were aged 19–24 months (6/28), compared with those infected with serotype 4 (1/18). The age

Table 1. Age distribution of rotavirus serotypes in hospitalised children with acute diarrhoea (Yogyakarta, Indonesia)

Serotype	No. of infected children at age (months)			
	0–6	7–12	13–18	19–24
1	0	0	1	0
2	0	3	0	2
3	3	13	6	6
4	5	6	6	1
Not typable	8	25	9	5

distribution of children from whom the rotavirus serotype could not be determined was similar to that of children where a serotype could be assigned.

An electropherotype pattern had been previously determined on 50 of the rotavirus specimens that were also serotyped [1]. These comprised 11 short pattern strains and 39 long pattern strains. One short pattern strain (A) was identified as serotype 2, and was the only known short pattern strain (1/11) typable in our assay. Long pattern strains D and E were identified as serotype 3 (16/39) or serotype 4 (10/39) respectively. A serotype could not be assigned to 13/39 (33%) of long pattern strains. No detectable electropherotype differences could be seen between long pattern strains identified as serotype 3 and those identified as serotype 3 × 1. Serotype 3 × 1 strains revealed only 11 bands after gel electrophoresis, and there was no evidence that these specimens contained mixtures of rotavirus strains. There was insufficient specimen containing the strain reacting with serotype 3 and 4 monoclonals to determine whether this was a mixture of two serotypes.

Discussion

During the period studied (June 1978–August 1979) all four major human serotypes (1, 2, 3, and 4) were present in Yogyakarta. Serotype 1 was identified in only one specimen. Serotypes 2, 3, and 4 were identified in 9, 53, and 36% respectively of typable specimens. The temporal distribution of serotypes 2, 3, and 4 varied throughout the fifteen months and could be correlated with climatic changes. These results resemble those from a recent survey of serotypes in infants hospitalized in Venezuela, which also detected all four major human serotypes and revealed temporal changes in rotavirus serotypes over a 15 month period [10]. Initially serotypes 2, 3, and 4 were approximately equally common (30, 33, and 38% respectively), but five months after beginning the survey serotype 1 strains comprised 75% of the typable strains excreted by Venezuelan children. The relative absence of serotype 1 strains from Yogyakarta during more than 12 months (at least in children admitted to hospital with rotavirus disease) is surprising, and emphasizes that successful vaccine strategies will require strains capable of protecting against all human serotypes.

Serotype 3 strains identified in Yogyakarta showed intratypic antigenic variations already described for serotypes 1 and 4 [5, 12]. Seven of the 17 serotype 3 strains reacted positively with a Mab previously shown to be cross-reactive between the VP_7 of serotypes 1 and 3. This reaction, although positive according to the criteria of our test, is reduced 10–100 fold compared with the homologous serotype 1-Mab 1 reaction [7]. Strains reacting with more than one serotype-specific monoclonal were identified in Venezuela, but were attributed to mixed infections [10]. The reaction observed with the Yogyakarta strains was not due to mixed infections (as judged from RNA patterns) but was due to possession of a neutralization site on VP_7 that is shared between serotypes 1 and 3. Similar strains have been identified infecting newborn babies in Melbourne [7].

We were unable to determine a serotype in 52/111 (47%) specimens even though 51 of them reacted strongly with the group specific (VP_6) monoclonal antibody. The high numbers of nontypable specimens may have been due to difficulties experienced in storage and transport of specimens from Yogyakarta to Melbourne that resulted in loss of the outer capsid layer. Electron microscopy (in 1978–79) had shown abundant rotavirus particles that were predominantly single shelled in most specimens.

It proved more difficult to serotype specimens previously shown to have a short electropherotype than those shown to be long-pattern strains. This may indicate that the VP_7 of serotype 2 is more vulnerable to the effects of gut enzymes during storage than the VP_7 antigens of serotypes 3 and 4. It is also possible that our relative failure to serotype short-pattern strains indicates the existence of antigenic variants of the VP_7 epitope of serotype 2 that are not recognized by our monoclonal antibody. Intratypic variations have already been described within serotypes 1 and 4 [5,12], but have not been described (to date) within serotype 2.

A new human serotype described as having a "supershort" electropherotype [14] was previously described in two Yogyakarta children enrolled in January 1979 [2]. Our serotyping assay did not include a monoclonal antibody specific for this new serotype. None of the non-typable strains in the present study possessed this characteristic electropherotype, and it is unlikely that further undetected infections with super-short rotavirus strains occurred during the period of this survey.

Overall, our results confirm that the relative frequency of rotavirus serotypes in a particular geographical area is variable over a period of time. Changes in the predominant serotypes can be related to climatic changes, even in a tropical country with no cold season. The relative absence of serotype 1 strains as a cause of severe disease in Yogyakarta indicates that vaccine strategies should not be based on the assumption that serotype 1 strains are persistent in all communities. Our results confirm the need for long-term sequential studies of the serotype of strains in various geographical areas in order to determine the relative frequency and temporal distribution of serotypes in hospitalized children in developed and developing countries. Once this knowledge is obtained it should be possible to devise rational vaccine strategies.

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