Rotavirus Epidemiology in Vellore, South India: Group, Subgroup, Serotype, and Electrophoretype

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Rotaviruses were detected in 163 of 916 (17.8%) specimens collected from children under 3 years of age with gastroenteritis in Vellore, South India, between August 1983 and July 1985. Rotaviruses were detected throughout the study period, with a peak prevalence in December to February (winter) and June to August (southwest monsoon season). A total of 117 rotavirus strains were tested for subgroup, serotype, and rotavirus double-stranded RNA electrophoretic migration pattern; 24.8% of the strains tested were subgroup 1, 69.2% were subgroup II, and 6.0% were neither subgroup 1 nor subgroup II. Subgroup I and II strains were circulating concurrently throughout the study. Of the 117 rotavirus strains, 32 (27.4%) were serotyped; 15 were serotype 1, 3 were serotype 2, 2 were serotype 3, and 12 were serotype 4. Three serotypes were circulating concurrently during the periods of peak rotavirus prevalence. In 100 of the 117 strains (85.4%) an RNA pattern was detected. One unusual subgroup I group A rotavirus with a long migration pattern and four atypical rotaviruses serologically related to group C were also detected.

Rotavirus is the most common pathogen detected in cases of infantile diarrhea in many parts of the world (15). It has been estimated that 5,000,000 deaths each year are caused by diarrhea in children under 5 years of age and that rotavirus may be associated with 20% of them (9). The World Health Organization is actively encouraging the development of vaccines to control rotavirus infections, and two candidate vaccines have already undergone clinical trials (11, 14). To develop the best strategy for using these vaccines it is necessary to understand the antigenic diversity of rotaviruses in different communities.

Three important rotavirus antigens have been described: the group antigen associated with the major inner capsid protein, designated VP6 (molecular size, 45 kilodaltons), the subgroup antigen also associated with the VP6 protein, and the serotype antigen associated with the two major outer capsid proteins VP7 (molecular size, 35 kilodaltons) and VP3 (molecular size, 80 to 88 kilodaltons). The contribution of these antigens to immunity to rotavirus infection is not yet completely clear, but experiments with animals suggest that serotype specificity is important (12, 24, 25).

Most epidemiological studies of rotavirus have been based on the detection of the common group antigen by an enzyme-linked immunosorbent assay (ELISA) or on the observation of rotavirus particles by electron microscopy (EM) (15). More recent epidemiological studies have been based on variations in the pattern of rotavirus double-stranded RNA genome segments in polyacrylamide gel electrophoresis (PAGE) (10) and on subgroup variations. However, there have been very few studies of serotype variations in different communities, owing to the lack of a suitable assay.

We report here an analysis of the rotavirus strains detected in Vellore, South India, over 2 years (1983 to 1985) in cases of diarrhea in children under 3 years of age. Vellore is a town of 300,000 inhabitants, but the hospital draws patients from a larger surrounding community. Vellore is situated 140 km inland from Madras at a height of 900 ft (274.32 m) above sea level; the climate is notable for its extreme dry heat in summer and two unreliable monsoons. Samples were tested for group, subgroup, and serotype antigens by ELISA, examined by EM, and analyzed by PAGE.

MATERIALS AND METHODS

Fecal specimens. Specimens were collected from children between 1 and 35 months old at the Child Health Department, Christian Medical College Hospital, Vellore, South India. Case patients were selected by proportionate, random sampling from all patients who had diarrhea lasting less than 72 h, who had not received any antibiotic or antiparasitic treatment in the 10 days before sample collection, and who had no complicating illness. Case patients were selected at the rate of 8 to 12 per week, evenly distributed through the age range, and the collections took place from August 1983 to July 1985. A total of 916 case patients were included in the study from the over 2,000 patients who fulfilled the study criteria. The prevalence of other viral and bacterial pathogens and the clinical aspects of this study will be described in detail elsewhere (M. Mathan, submitted for publication).

Detection of rotavirus by EM. An approximately 10% suspension of each fecal specimen was made in phosphate-buffered saline by vigorous homogenization in a vortex mixer for 2 to 3 min. The suspension was then clarified (10,000 × g for 30 min), and the supernatant was pelletted at 50,000 × g for 90 min. The pellet was suspended in 1 or 2 drops of distilled water, placed on a Parlodion-coated grid, and negatively stained with 3% ammonium molybdate. The grids were examined with a Phillips EM 200. Immune EM studies for the confirmation of group C rotavirus were performed by the method of Cubitt et al. (8). Guinea pig serum 444 used in the immune EM studies was raised against a group C human rotavirus (5066) and had previously been shown to be group C specific (5).

Detection of rotavirus by ELISA. An approximately 10% suspension of each fecal specimen was made in phosphate-
buffered saline and clarified by low-speed centrifugation (2,000 × g for 10 min). Rotaviruses were detected with a group A-specific ELISA based on polyclonal sera (3) provided by the World Health Organization Reference Laboratory, East Birmingham Hospital, Birmingham, United Kingdom.

**Detection of rotavirus double-stranded RNA by PAGE.** Rotavirus double-stranded RNA was extracted from 0.5 ml of 10% fecal suspensions, and PAGE was performed as previously described (22).

**Subgrouping and serotyping of rotavirus strains.** For both subgrouping and serotyping, an NADP-enhanced enzyme immunoassay was used (2). Briefly, polystyrene microtiter plates (Nunc) were coated with a 1/10,000 dilution of serum from a rabbit which had been hyperimmunized with a mixture of rotavirus isolates representing serotypes 1 to 4. The plates were kept overnight at 4°C, and the wells were emptied.

Stool samples (100 μl; 10 to 20% [vol/vol]) extracted in 0.1 M Tris-buffered saline (pH 7.5) containing 0.1% (vol/vol) Tween and 3% (wt/vol) bovine serum albumin (TBS/T/BSA) were added to each of 16 wells in pairs across plates. The plates were kept at 4°C overnight and washed six times with TBS/T.

Monoclonal antibodies as ascitic fluids were diluted 1/10,000 in TBS/T/BSA, and 100 μl was added to two wells for each antibody; eight different monoclonal antibodies were used on each plate (A, SG1, SGII, 60, RV 4:2, RV 5:3, RV 3:1, and ST3). (Monoclonal antisera RV 4:2, RV 5:3, RV 3:1, and ST3 were generous gifts from B. Coulson. These monoclonal antibodies are serotype specific and represent serotypes 1 to 4, respectively [7]. Monoclonal antisera 60, SG1, and SGII were generous gifts from H. Greenberg and R. D. Shaw [21]. Antibody 60 is cross-reactive with VP7 of different serotypes and was used as a control for complete particles.) The plates were incubated for 2 h at 37°C and washed.

Anti-mouse polyclonal gamma globulin-alkaline phosphatase conjugate (Sigma Chemical Co.) was diluted 1/2,000 in TBS/T/BSA, and 100 μl was added to each well. The plates were incubated for 1.5 h at 37°C and washed six times with TBS/T.

NADP-substrate (100 μl; IQ Bio Ltd., Cambridge, United Kingdom) was added to each well, and the plates were left at room temperature (approximately 22 to 24°C) for 15 min. The plates were not washed. Ethanol-INT violet amplifier solution (200 μl; IQ Bio) was added to the 100 μl of NADP-substrate. Great care was taken not to contaminate adjacent wells. The amplifier was added in the same order as the substrate, and approximately the same time was taken to do this. The reaction was stopped with 3 M H₂SO₄ after 15 min. Optical densities were read at 492 nm.

Samples were considered to give a positive result with any serotype-specific monoclonal antibody if the optical density obtained was at least 2.5 times the value of the average optical densities obtained with the other antibodies.

**RESULTS**

**Seasonal variation of rotavirus.** Rotavirus was identified in 163 (17.8%) of the 916 case patients with diarrhea examined over 2 years (Fig. 1). The prevalence varied between 2.7% in November 1983 and 32% in February 1985. Two peaks of rotavirus prevalence were detected in both years of the study: (i) in December to February, the coolest period of the year, following the northeast monsoon in October and November, and (ii) in June to August, the season of the southwest monsoon and a hot and humid time of the year.

**Detection of rotavirus by EM and ELISA.** A total of 163 rotavirus-positive samples were detected by either EM or ELISA for the group antigen. Rotavirus was detected in 130 (79.8%) of the specimens by both methods, in 27 specimens (16.5%) by EM alone, and in 6 specimens (3.6%) by ELISA alone. The six ELISA-positive, EM-negative specimens were all confirmed as rotavirus by the subgrouping ELISA (two subgroup I and four subgroup II strains) and by PAGE (two short and four long electrophoretic migration patterns). Of the 27 specimens positive for rotavirus by EM but negative by ELISA, sufficient material was available from 10 specimens for detailed analysis at a second laboratory (World Health Organization Reference Laboratory, East Birmingham Hospital). Of these 10 specimens, 4 gave positive group and subgroup results on testing by the NADP-amplified ELISA and therefore represent false-negative ELISA results in Vellore; of the 10 specimens, 2 were negative by EM, ELISA, and PAGE, and 4 were confirmed as EM positive, PAGE positive, and ELISA negative. The electrophoretic patterns of two of these EM-positive,
antiserum to group C rotavirus when tested by immune EM. Of the 17 specimens which were EM positive and ELISA negative in Vellore but which were not tested further, only one rotavirus particle was seen in 6 by EM, and in four others the rotaviruses seen were aggregated, indicating that host antibody might be blocking the ELISA reaction.

Subgrouping, serotyping, and electropherotyping of rotavirus isolates. A total of 117 of the rotavirus-containing specimens identified in this study were examined in detail. These were all selected from the EM-positive, ELISA-positive group. The resulting monthly distribution of these 117 strains by subgroup and serotype is shown in Fig. 3. A total of 29 strains were subgroup I (24.8%), 81 were subgroup II (69.2%), and 7 (6.0%) were neither subgroup I nor subgroup II. Both subgroup I and subgroup II strains were found to be circulating concurrently throughout the study. Only 32 of 117 strains (27.4%) could be serotyped, and of these, 15 (46.9%) were serotype 1, 3 (9.3%) were serotype 2, 2 (6.3%) were serotype 3, and 12 (37.5%) were serotype 4. A further 14 samples (11.9%) reacted positively with a VP7-specific monoclonal antibody but not with the four serotype-specific monoclonal antibodies. Of these 14 specimens, 12 contained detectable amounts of RNA (10 short and 2 long patterns). None of them showed an unusual distribution of RNA segments. A total of 11 were typed as subgroup I, and 3 were typed as subgroup II. Five subgroup I strains were identified in August 1984, and the other nine strains were distributed evenly throughout the period of the study. A total of 71 strains showed no reaction with any of the monoclonal antibodies used.

Of the 117 specimens, 100 (85.4%) contained sufficient RNA for PAGE; 75 had long patterns, and 25 had short patterns. Of the 81 subgroup II rotaviruses, 71 (87.7%) had detectable RNA, and all of these had long patterns. Of the 29 subgroup I rotaviruses examined, 25 (86.2%) contained detectable RNA; 24 had short patterns and 1 had a long pattern. Of the seven rotaviruses that could not be subgrouped, three had long patterns and 1 had a short pattern.

**DISCUSSION**

Several different epidemiological patterns of rotavirus infection have been described, and these vary with the
nature of the community and the climate of the area studied. In general, studies in temperate climates have described a winter peak, whereas those in tropical areas have described a more uniform distribution throughout the year (15, 19). Vellore is situated on latitude 13°N, and although rotaviruses were detected throughout the year, two peaks of rotavirus prevalence were detected, one in June to August, coinciding with the southwest monsoon, and one in December to February, associated with the cooler temperatures of winter. These findings confirm the prevalence of rotavirus (16), although coinciding and typing rotaviruses by neutralization, been very (17,8%) were and they of the dom serotyping sensitivity samples under the be and they should be transported without the rotavirus, preferably ing that immunity rotavirus vaccine This suggestion in infection with, different infants situation rotavirus disease react with the the community may 1
Previous studies of low suggest as asymtomatic, success in both studies. Evidence demonstrates three 10% as asymptomatic, illness in large, rotavirus vaccine may perceived on July 9, 2017 by guest http://jcm.asm.org/ Downloaded from http://jcm.asm.org/ Downloaded from http://jcm.asm.org/