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Rotavirus Serotypes and Subgroups in Gastroenteritis

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Summary

Omotade OO, Olaleye OD, Oyejide CO, Avery RM, Pawley A and Shelton AP. Rotavirus Serotypes and Subgroups in Gastroenteritis. *Nigerian Journal of Paediatrics 1995; 22: 11.* Faecal specimens from 66 children, aged between one month and 39 months, presenting with gastroenteritis at three hospitals in Ibadan, were examined by electron microscopy for viral agents. Rotavirus particles were identified in 24.2 percent of the specimens and further analysis of these rotavirus-positive specimens by SDS-PAGE and nicotinamide-adenine-dinucleotide-phosphate (NADP) enhanced ELISA, showed that all the patients were infected with serotype I of group A rotavirus. Of the 13 rotavirus positive specimens, 15.4 percent belonged to subgroup I, while 84.6 percent of the 11 specimens belonged to subgroup II. Diarrhoea, vomiting and dehydration were associated with most of the 66 cases. In addition, blood-stained faeces was found in 25 percent of those with rotavirus, while mucoid faeces was found in 72 percent of those without rotavirus.

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Introduction

INFECTIOUS gastroenteritis is a major cause of morbidity and mortality among children throughout the world[1] and viruses constitute most of the organisms associated with this diarrhoeal disease. In developing countries, several workers have reported that rotavirus infection is an important cause of childhood death associated with diarrhoea.[2-4] Hospital-based studies in Nigeria have shown that between 3.6 percent and 20.1 percent of diarrhoeal diseases are associated with rotavirus infection,[4] while a community-based study has

also shown that 7.7 percent of childhood diarrhoeas are associated with rotavirus infection.[5] Although it has been shown that rotavirus is important in the aetiology of childhood diarrhoeas in the country, the serotypes of this virus are unknown. The development of any useful vaccines either locally, or from elsewhere for use in the country must take the rotavirus serotypes into consideration before local utilization of such vaccines. As of now, and to our knowledge, rotavirus vaccines though available in developed countries, are not yet available locally. Thus, typing the serotypes of the local rotavirus would be a useful tool in deciding the usefulness or otherwise, of any intended vaccine production. It would also indicate the serotypes on which our scientists should concentrate in the development of any vaccines. The aim therefore, of the present study was to determine the local rotavirus serotypes and subgroups that are associated with childhood gastroenteritis.

Materials and Methods

Sixtysix faecal specimens were collected from convenience-sample selected children, aged between one month and 39 months, who attended three major hospitals in Ibadan, (University College Hospital, Adeoyo State Hospital and Oni Memorial Children's Hospital, respectively) from January to July 1989, for treatment of diarrhoea. The need to sample conveniently arose because of the necessity of keeping the samples frozen after collection.

The specimens were stored and kept frozen in Nunc tubes at 4°C after the collection and later transported by air in ice, to the University of Nottingham Teaching Hospital, United Kingdom, for laboratory analysis. Demographic data and clinical features, including the feeding pattern of the children, were recorded by attending paediatricians, using standardized forms designed for the study.

All the specimens were examined initially by electron microscopy (EM), using negative-staining technique as described by Flewett *et al.*[6] All positive specimens for rotavirus particles were further analysed for rotavirus electrophoretypes using polyacrylamide gel electrophoresis (PAGE). Specimens were prepared as described by Moosai, Carter and Madely; [7] 100µl of 10 percent (w/v) sodium dodecyl sulphate (SDS) and 10 percent faecal supernatant were mixed and incubated for 30 minutes at 37°C. Thereafter, 200µl of sample buffer was added and the extracted and purified RNA was used for gel electrophoresis. Electrophoresis was performed, using a discontinuous gel [8] consisting of a lower separating gel and an upper stacking gel, cast in Protea II slab (Bio-Rad laboratories, Hertfordshire, UK), according to the manufacturer's instructions. Gels were stained with silver nitrate as described by Herring *et al.* [9] Determination of electrophoretype was done by comparing the distance of migration of the 11 segments of the rotavirus RNA genome for bovine virus RNA control with each specimen. The distance of migration of the buffer/dye front was also recorded. Specimens in which rotavirus particles were seen under the EM were also examined by the nicotinamide-adenine-dinucleotide-phosphate (NADP) enhanced enzyme-linked immunosorbent assay (ELISA) to determine the subgroups and the serotypes of the rotavirus present.[10] Data were analysed, using Wilcoxon Rank Sum [11] test to assess the significance of observed differences.

Results

The age of the children with rotavirus infection ranged from two to 39 months and from one month to 39 months for those without the infection. There was no significant difference in the median age of the two groups (P>0.05). Significantly, 36 (72.0 percent) of 50 patients without rotavirus, had mucoid faeces, but none of those with rotavirus had mucoid stools. Four (25 percent) of 16 children with positive rotavirus, had blood-stained stools, while those with negative rotavirus did not have blood-stained faeces (P< 0.005). There was no difference in the number of days spent before presentation at the hospital between the two groups (Table). The feeding pattern depended on the ages of the children and no difference was observed between those with and those without rotavirus infection. There was no difference in the occurrence of fever, vomiting and the number of bowel motions between the groups (P>0.5).

Analysis of the treatment given to the children by the mothers before presentation in hospital (Table) showed that in 32 percent of those who were virus-negative and 25 percent of those who were virus-positive, no medication was given before presentation. In 56.3 percent of the virus-positive and 38 percent of the virus-negative children, drugs alone were used before hospital attendance; 12.5 percent of the virus-negative and 20 percent of the virus-positive children used oral rehydration solution (ORS) only, while 9.1 percent of the two groups used both ORS and medication before attending the hospital. Rotavirus particles (Fig) were detected by EM in 16 (24.2 percent) of 66 faecal samples. No unusual electrophoretypes were present, while significant polymorphism was observed as previously reported.[11] All the identified rotaviruses belonged to group A, serotype I. Thirteen (81.3 percent) of the 16 rotavirus-positive stool specimens were subgrouped and two (15.4 percent) of these belonged to subgroup I, while the other 11 (84.6 percent) belonged to subgroup II. No unusual subgroup/serotype associations occurred.

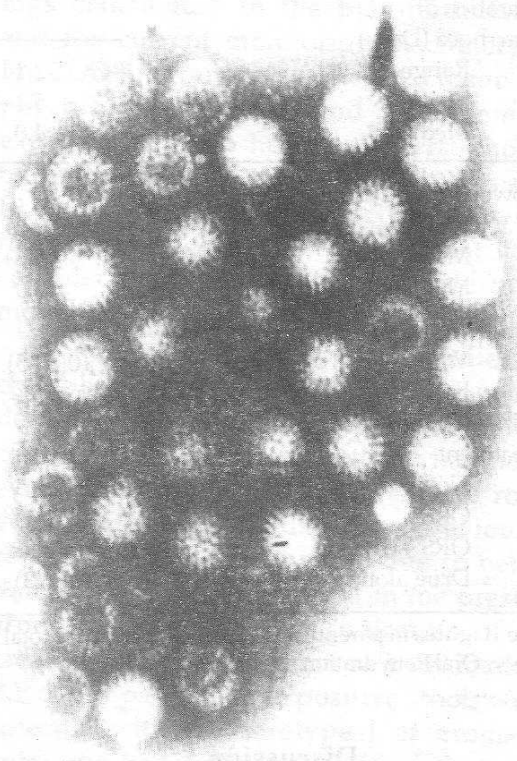

Fig. Electron micrograph showing rotavirus particles in the stool supernatant of a child with gastroenteritis (magnification X 125,000)

TABLE

Clinical Features of Children with and of Those without Rotavirus Gastroenteritis

Feature	Rotavirus		Total (n = 66)
	Negative (n = 50)	Positive (n = 16)	
Type of Feeds			
Breastmilk only	5(31.3)	16(32.0)	21(31.8)
Artificial feeds only	3(18.7)	1(2.0)	4(6.1)
Breast + artificial feeds	6(37.5)	21(42.0)	27(40.9)
Weaning diet	-	4(8.0)	4(6.1)
Adult diet	2(12.5)	8(16.0)	10(15.1)
Duration of diarrhoea (Days)	Days		
Range	2-14	2-14	
Mean	5.1+3.0	4.0+1.8	
Median	4.0	4.0	
Bowel motions/Day			
Range	3-12	3-8	
Mean	5.6+2.6	4.6+1.8	
Median	5.0	4.0	
Other symptoms			
Vomitting	10(62.5)	29(58.0)	39(59.1)
Fever	12(75)	39(78.0)	51(77.3)
Initial home treatment			
None	4(25)	16(32.0)	20(30.3)
ORS	2(12.5)	10(20.0)	12(18.2)
ORS + drug	1(6.2)	5(10.0)	6(9.1)
Drug alone	9(56.3)	19(38.0)	28(42.4)

Note: Figures in parentheses represent percent of total
ORS = Oral Rehydration Solution

Discussion

Rotavirus causes gastroenteritis among all age groups, but most severe outbreaks have been reported in neonates and young children.[1] Human rotavirus has been classified into three groups on the basis of internal antigens[12] and group A is most frequently associated with childhood gastroenteritis.[13] There are four serotypes of group A, based on the virus surface pro-

tein believed to be the major neutralization antigen.[14] Sequential infections with different serotypes have been reported in children,[15] but it is not fully known whether cross-protection with antibodies to different serotypes occurs. Information on rotavirus serotypes in different geographical regions is important for vaccination programmes, because the degree of protection afforded by monovalent vaccines vary greatly.[16]

The clinical picture of gastroenteritis in the present study was similar to that previously described for rotavirus infection elsewhere.[1] However, the mean number of bowel motions per day among those positive for raotavirus was not significantly different from those that were negative. This finding may be related to the fact that all the children were outpatients without any easy method of verifying all the statements obtained from the mothers. Previous studies have revealed a longer duration of diarrhoea among children with rotavirus infections.[4 5] The mean number of days before our patients attended the hospital was long and this would suggest that parents utilized alternative sources of care before bringing their children to the hospital. It should be noted that 27.3 percent of all the cases had received oral rehydration solution before coming to the hospital, a percentage far below the recommended target for the Control of Diarrhoeal diseases (CDD) Programme.[17] Similarly, 56.3 percent of those positive and 38 percent of those negative for rotavirus had received antidiarrhoeal medication without a proper diagnosis of the aetiology of the diarrhoea. This is an arbitrary as well as an inappropriate use of medication that is contrary to the ideals of the CDD, which restrict the use of medication to only proven cases that require such medications. It should be observed however, that the positive cases for rotavirus also had blood in the faeces, a condition that satisfied the stringent ideals of the CDD. This probably accounted for the high medication usage in this group. Even among patients without blood in the faeces (the rotavirus-negative group), 38 percent used medication alone, before coming to hospital. Similar previous observations to the present findings contributed to the plan-formulation and the current monitoring machinery of the CDD Programme.[17] The need to modify parental perceptions and management expectations from health workers, should be re-examined in the light of the present findings, as parents may oscillate between health facilities in search of what they consider important and appropriate treatment for childhood diarrhoeal diseases.

Previous epidemiological studies of childhood gastroenteritis had shown endemic presence of the rotavirus in the country.[5 18] The present study has however revealed a higher prevalence of rotavirus infection in Ibadan than previously reported.[4 5 19] This may be due to better detection methodology used in the present study, or it may be an indication of increased rotavirus infection rate in Ibadan. All of our patients with positive stools were infected with the serotype I of group A rotavirus and futher analysis of the samples showed that subgroup II rotavirus was commoner than subgroup I. This finding is similar to that of a higher frequency of subgroup II in other parts of the world.[1]

Although the full clinical significance of different rotavirus subgroups is unknown, subgroup II rotaviruses have been associated with a higher rate of hospitalization, more severe symptoms and higher occurrence of respiratory symptoms.[1] Because the subjects in the present study were all outpatients, we were unable to fully correlate the severity of symptoms with the rotavirus subgroups. Although only serotype I was detected in all the faecal specimens positive for rotavirus in the present study, it is possible that other serotypes were co-infecting, but to a lesser extent, as shown by others.[20 21] It is therefore, suggested that nation-wide studies be undertaken in order to determine the rotavirus serotypes that exist in different parts of the country before any vaccination programme is embarked upon.

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