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Subgroups, Serotypes, and Electrophoretypes of Rotavirus Isolated from Children in Bangui, Central African Republic

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The subgroups and serotypes of 178 strains of rotavirus isolated from diarrheic and healthy children in Bangui, Central African Republic, during a 27-month period were determined by enzyme-linked immunosorbent assay. The subgroup was determined for 152 of the viral strains, 18.4% being subgroup I and 81.6% being subgroup II. Of the 143 strains which could be serotyped, 71.3% were serotype 1, 15.4% were serotype 2, and 13.3% were serotype 3. Serotypes 1 and 3 were detected throughout the study, while serotype 2 was detected only during 8 months. No serotype exhibited any special epidemiological properties. The serotypes were found to consist of three different electrophoretypes, two long ones (A and B) and a short one (C). All subgroup I, serotype 2 strains presented short electrophoretypes. Strains with identical long electrophoretypes A were either serotype 1 or serotype 3.

Since their discovery by electron microscopy in duodenal biopsies in Melbourne, Australia, and from stools of diarrheic children in Birmingham, United Kingdom (4, 9), rotaviruses have been shown to be the major cause of infantile gastroenteritis in developed and developing countries (5, 8, 18, 30). Most rotaviruses isolated from humans have a common antigen which can be detected by a number of serological tests (35). These isolates, along with others from animal hosts, are called group A rotavirus. Recently, rotaviruses which lack this antigen and therefore do not react in conventional serological tests have been isolated from humans and animals (6, 7, 20, 23, 26, 32). These atypical rotaviruses are designated groups B, C, and so forth (24).

Apart from the group antigen, group A rotaviruses carry two major distinct antigenic specificities. The first is associated with the major inner capsid protein designated Vp6, with a molecular weight of approximately 45,000, and defines the subgroup (16, 17, 31, 33, 38). The second antigenic specificity is associated with the major outer capsid glycoprotein Vp7 (molecular weight, approximately 35,000) and is involved with neutralization (3, 17, 36). The term serotype is used to define this specificity, and at least four distinct serotypes and two subgroups of rotavirus isolated from humans have been defined (29). The enzyme-linked immunosorbent assay (ELISA) has been used to investigate both the subgroup and serotype antigens (28, 33).

The genome of rotaviruses consists of 11 segments of double-stranded RNA which can be separated by polyacrylamide gel electrophoresis. Such electrophoresis allows comparisons to be made between different strains (27).

Apart from minor differences in the molecular weights (and, hence, migration patterns) of individual segments from different isolates, two major electrophoretypes have been described, designated long and short. It has been shown that for rotaviruses from humans these patterns correlate with subgroup specificities, the long pattern corresponding to subgroup II and the short pattern corresponding to subgroup I. This is not the case for rotaviruses isolated from animals (15, 25). Similarly, major differences in electrophoretypes have been demonstrated between rotaviruses from different groups, and most atypical rotaviruses were originally detected by polyacrylamide gel electrophoresis (6, 7, 20, 24, 26, 32). Therefore, both serological and molecular methods can be used to characterize rotavirus strains (27).

An earlier study conducted in Bangui showed that rotavirus was the most common enteropathogen identified in the stools of children under 2 years old with diarrhea (12). Another study of 61 strains of rotavirus isolated in Bangui showed that only two electrophoretypes circulated in the community during 12 months (11).

We report here the results of subgrouping, serotyping, and electrophoretyping of 178 strains of rotavirus isolated in Bangui, Central African Republic.

MATERIALS AND METHODS

All samples were stools from diarrheic or nondiarrheic children under 5 years old living in Bangui during January 1983 to April 1985. The nondiarrheic children were healthy children attending a primary health care center or belonging to a cohort of children monitored from 0 to 2 years of age for rotavirus infections. The presence of rotavirus in the stools was detected by ELISA with the World Health Organization test provided by the Birmingham laboratory (2).

TABLE 1. Results of subgrouping and serotyping tests

Serotype	No. of strains		
	Subgroup I	Subgroup II	Low antigen
1	0	102	0
2	22	0	0
3	0	19	0
Not typable	6	3	26

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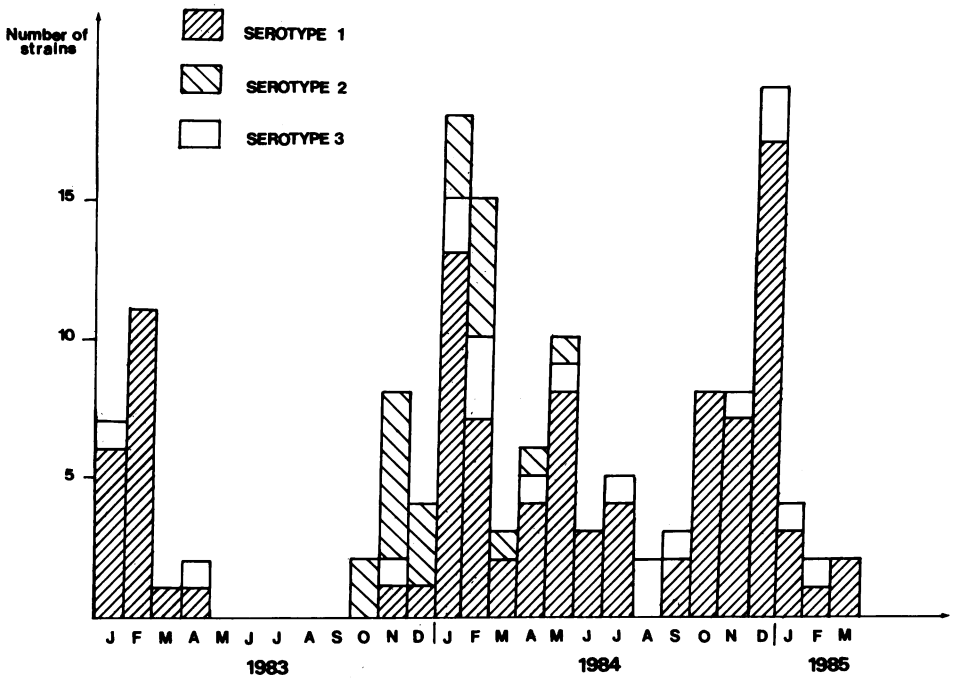

FIG. 1. Monthly distribution of the three serotypes.

Subgrouping and serotyping. Both subgroup analysis and serotyping were performed by ELISA. The subgroup assay was performed as described previously (10) with monoclonal antibodies kindly provided by H. Greenberg (13). The serotyping assay is described elsewhere (G. M. Beards, J. Virol. Methods, in press). Briefly, samples were tested by ELISA with serotype-specific monoclonal capture and/or detecting antibodies. The serotype-specific monoclonal antibodies were kindly provided by H. Greenberg and R. Shaw (28).

Polyacrylamide gel electrophoresis of genomic RNAs. The electrophoresis of rotaviral RNAs was performed by the method described by Herring et al. (14). Briefly, the viral RNA was extracted from fecal suspensions with a phenol-chloroform mixture, precipitated in ethanol, and dissolved in Laemmli sample buffer (19). After electrophoresis at 60 mV for 16 h, the gel was stained with ethidium bromide in aqueous solution (1 μg/ml) or with silver (14).

RESULTS

A total of 178 samples were positive for rotavirus by the World Health Organization ELISA and were tested for subgroup and serotype specificities and electrophoretype.

TABLE 2. Distribution of three serotypes among diarrheic and control children

Serotype	No. (%)	
	Diarrheic children (n = 140)	Controls (n = 38)
1	77 (55.0)	25 (65.8)
2	18 (12.9)	4 (10.5)
3	16 (11.4)	3 (7.9)
Not typable	29 (20.7)	6 (15.8)

Subgroup analysis. Subgroup analysis was successful for 152 of the 178 samples tested (85.4%); 26 samples had low antigen levels and could not be subgrouped (or serotyped) with any confidence. The results of subgroup determination are summarized in Table 1. Of the 152 strains, 28 (18.4%) were subgroup I and 124 (81.6%) were subgroup II.

Serotyping. Of the 152 subgrouped rotavirus strains, 143 were serotyped (Table 1). Of the 28 subgroup I strains, 22 were found to be serotype 2; 6 were not typed, possibly as a result of loss of outer capsid proteins. Of the 124 subgroup II strains, 102 were serotype 1, 19 were serotype 3, and 3 were

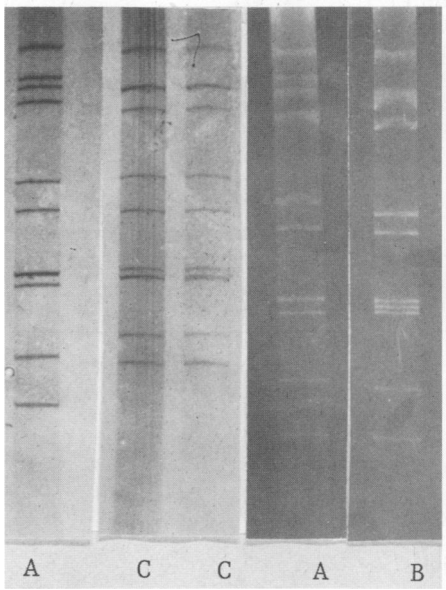

FIG. 2. Electrophoretypes of rotavirus identified in Bangui. Lanes are labeled according to electrophoretype.

TABLE 3. Repartition of three serotypes in the different groups

Serotype	No. (%) of strains from children aged:			
	0–6 mo	6–12 mo	1–5 yr	Total
1	46 (55.4)	38 (56.7)	18 (64.3)	102
2	9 (10.8)	10 (14.9)	3 (10.7)	22
3	10 (12.1)	7 (10.5)	2 (7.1)	19
Not typable	18 (21.7)	12 (17.9)	5 (17.9)	35
Total	83	67	28	

neither serotype 1, 2, nor 3. (Testing for serotype 4 specificity was not performed, owing to a lack of suitable sera.)

The distribution of the three serotypes among diarrheic and control children is summarized in Table 2 and for the different age groups in Table 3. There was no apparent difference between the three serotypes in their prevalence in symptomatic or asymptomatic children or in the different age groups. Figure 1 shows the incidence of each serotype for each month and year of the study. Serotype 2 was detected during a short period of 8 months from October 1983 to May 1984 only. The two other serotypes, however, were detected throughout the 27 months.

RNA polyacrylamide gel electrophoresis. Three different RNA migration patterns were observed, designated A, B, and C. These are shown in Fig. 2: A and B are long electrophoretypes, while C is a short electrophoretype. The relationships between electrophoretypes, subgroups, and serotypes are shown in Table 4.

DISCUSSION

By using a monoclonal double-antibody sandwich ELISA, the subgroup specificities of 85.4% of 178 rotavirus-positive stool samples from children in Bangui were determined. Among them, the frequency of subgroup II samples was 81.6%, compared with 18.4% for subgroup I. These results correlate with those reported for other parts of the world, in that there was a greater preponderance of subgroup II than of subgroup I (21, 22, 34, 37).

Three serotypes were identified among the 143 typable strains. Serotype 1 was encountered more frequently than serotype 2 or 3. Serotypes 1 and 3 were identified throughout the study period, while serotype 2 was detected only during a short period.

TABLE 4. Distribution of the different electrophoretypes and their relation to subgroups and serotypes

Subgroup and serotype	No. of strains with electrophoretype:				
	A	B	C	Unknown	Total
I					
2	0	0	22	0	22
Not typable	0	0	5	1	6
II					
1	94	2	0	6	102
3	16	0	0	3	19
Not typable	3	0	0	0	3
Not groupable	20	0	0	6	26
Total	133	2	27	16	178

It does not appear that any one serotype was significantly more pathogenic than another, since the different serotypes were found in equal proportions in the diarrheic and control (asymptomatic) children. Also, none of them seemed to be associated with a single age group.

The electrophoretypes of 91% of the samples were determined. Interestingly, only three RNA migration patterns were observed. This low genomic diversity was observed previously in Bangui, in 1982. Then, only two patterns were obtained (11). One, designated B, was seen on only two occasions in this study. The second one, designated A, was most frequently seen during the 3 years.

The correlation of the short RNA pattern with subgroup I strains reported by other researchers was confirmed in this study (15).

Another interesting observation was the finding of rotavirus strains with identical electrophoretypes but different serotype specificities: indeed, of 110 strains with electrophoretype A (long electrophoretype), 94 were serotype 1 and 16 were serotype 3. This observation, reported previously for long electrophoretypes, shows that electrophoretype cannot be used to predict serotype of any one strain, at least for long RNA patterns (1). This illustrates how serological and molecular techniques complement one another in epidemiological investigations of rotavirus diarrhea.

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