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Rotavirus Infection of Young Children in Two Districts of Kenya from 1982 to 1983 as Analyzed by Electrophoresis of Genomic RNA

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Employing techniques of polyacrylamide gel electrophoresis of viral RNA segments, we studied rotavirus strains and their relative contributions to rotavirus gastroenteritis epidemics in two major districts of Kenya. From early 1982 to the middle of 1983, 18 representative electropherotypes, including 6 short strains, were detected in 30 rotavirus specimens obtained from Nairobi, whereas 16, including 3 short strains, were detected in 70 virus specimens from coastal areas. With the exception of one strain, there were no identical electropherotypes between the two groups of rotaviruses obtained from these different districts. A change in predominant electropherotypes was observed in Mombasa in early 1983, and subsequently, newly occurring strains were detected in a small town along the coast when an apparent increase in gastroenteritis was observed in the district.

Rotaviruses have been established as the most important agents of acute gastroenteritis in infants and young children in many countries (3, 5, 8). Although studies carried out in developing countries to determine the relative importance of rotavirus infection among various types of infantile gastroenteritis have been rather limited, it is likely that the virus may play an appreciable role in such diarrheal illnesses. For example, Black et al. (2) have observed in Bangladesh that about half of the cases of gastroenteritis treated in young children are associated with rotavirus.

The situation in East Africa may be similar to this. A preliminary survey of children, particularly of those under 3 years old, who received treatment in two districts of Kenya (Mombasa and Nyeri) from 1981 to 1982 has revealed that up to 50% of these cases are due to rotavirus, with infection being detected almost every month in both areas (12). A similar observation was made by Mutanda (13) in a survey of several districts, including Nairobi. Thus, the results suggest an ubiquitous distribution of rotavirus and its important role as the major cause of gastroenteritis in young children in Kenya.

Recently, the existence of different types of human rotavirus has been demonstrated by immunological assays (6, 16, 17) and gel electrophoretic analysis of viral RNA (4, 5, 14, 15). It is, therefore, important to recognize prevalent strains and to understand their contributions to rotavirus gastroenteritis epidemics in more detail. For this reason, we initiated an electrophoretic analysis of the genomic RNA of rotaviruses obtained from two major districts of Kenya (coast and Nairobi) during the period of 1982 to 1983.

MATERIALS AND METHODS

Rotavirus specimens. Stool specimens of children under 3 years old who were acutely ill with gastroenteritis were collected from two districts (coast and Nairobi) and screened for the presence of rotavirus by an enzyme-linked immunosorbent assay (Rotazyme; Abbott Laboratories, North Chicago, Ill.). On the coast, this survey was initiated in July 1982 at the Coast Province General Hospital and the Mvita Health Clinic, both of which are located in Mombasa and cover mainly urban areas of the city. Until May 1983, patients with rotavirus infections were detected during all months studied, and the average incidence of infection in the two hospitals was 25%. A total of 55 rotavirus specimens from these two hospitals were subjected to analysis. In addition, 15 specimens were obtained from the Waa Dispensary (15 km south of Mombasa) and from the Kilifi District Hospital in Kilifi (50 km north of Mombasa) from October 1982.

In Nairobi, specimens were obtained from The Kenyatta National Hospital and from another private clinic in the city between January 1982 and May 1983. A total of 30 virus specimens were available for the study.

Purification of rotavirus and preparation of genomic RNA. Ten to 15 ml of a 20% stool suspension was prepared in phosphate-buffered saline, and crude materials were removed by low-speed centrifugation at 6,000 rpm for 30 min. The supernatant fluid was layered on the top of a 45% (wt/vol) sucrose cushion and centrifuged at 100,000 × *g* for 3 h. The resultant pellets were gently washed once with distilled water and suspended in an appropriate volume of 50 mM Tris hydrochloride-buffered saline (pH 7.2). The purified virus was disrupted with sodium dodecyl sulfate and 2-mercaptoethanol and then deproteinized with phenol as previously described by Espejo et al. (5). Ethanol-precipitated RNA was again dissolved in 10 mM Tris hydrochloride buffer with 1 mM EDTA at pH 8.0.

Polyacrylamide gel electrophoresis of RNA. Slab gels of 7.5% (wt/vol) acrylamide and 0.2% (wt/vol) bisacrylamide were prepared by the method of Laemmli (10), and 5 to 15 μl of RNA specimen mixed with 60% (wt/vol) sucrose was applied to the gel. Electrophoresis was carried out at 25 mA for 16 h at 4°C, and the gel was stained with 1 μg of ethidium bromide per ml in 10 mM Tris hydrochloride buffer (pH 7.5) before it was observed on a short-wave UV transilluminator. Final identification of viruses with similar electrophoretic migration patterns was determined by coelectrophoresis.

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TABLE 1. Segment variations in the genomic RNA of rotaviruses in three groups of electropherotypes, each with similar electrophoretic mobility

Group	Virus coelectrophoresed[a]	Segment variation
1	Cn + Dn	9, 10
	Cn + On	9, 10
	Cn + Nn	9, 10
	Dn + On	10
	Dn + Nn	10
2	Gn + Hc	1, 7, 10
	Gn + Ln	6
	Gn + Qn	1
	Hc + Ic	10
	Hc + Qn	7, 10
3	Kn + Ac	5, 6, 7, 10
	Kn + En	1, 2, 7, 8, 10
	Kn + Mc	1
	Mc + Sn	1, 6

[a] Lowercase letters indicate electropherotypes detected either in Nairobi (n) or along the coast (c).

RESULTS

Diversity of rotavirus genomic RNA. Up to May 1983, we analyzed 100 rotavirus specimens, of which 70 were obtained from the coast and 30 from Nairobi. The results were arranged according to two major electropherotypes (5, 7) which are generally accepted as long and short strains. In both areas, the majority of the viruses, 67 (95.7%) of the 70 strains from the coast and 22 (73.3%) of the 30 strains from Nairobi, were designated as long strains, although the viruses with electrophoretically slowly migrating 10th and 11th segments of RNA (short strains) were more frequently detected in Nairobi during this period (8 [26.7%] of 30 strains, as compared with 3 [4.3%] of 70 strains from the coast.)

The representative electrophoretic migration patterns of the genomic RNA of rotavirus from the coast by the order of detection are shown in Fig. 1a. Of these 16 strains, only 3 were short strains (D, E, and P). The results of strains from Nairobi are shown in Fig. 1b. Although the total number of virus specimens subjected to analysis was less than half of those in the coast area, 18 different strains, including 6 short ones (B, H, I, J, M, and P), were identified.

As suggested by these results, the genomic migration patterns of certain strains were similar, and therefore analy-

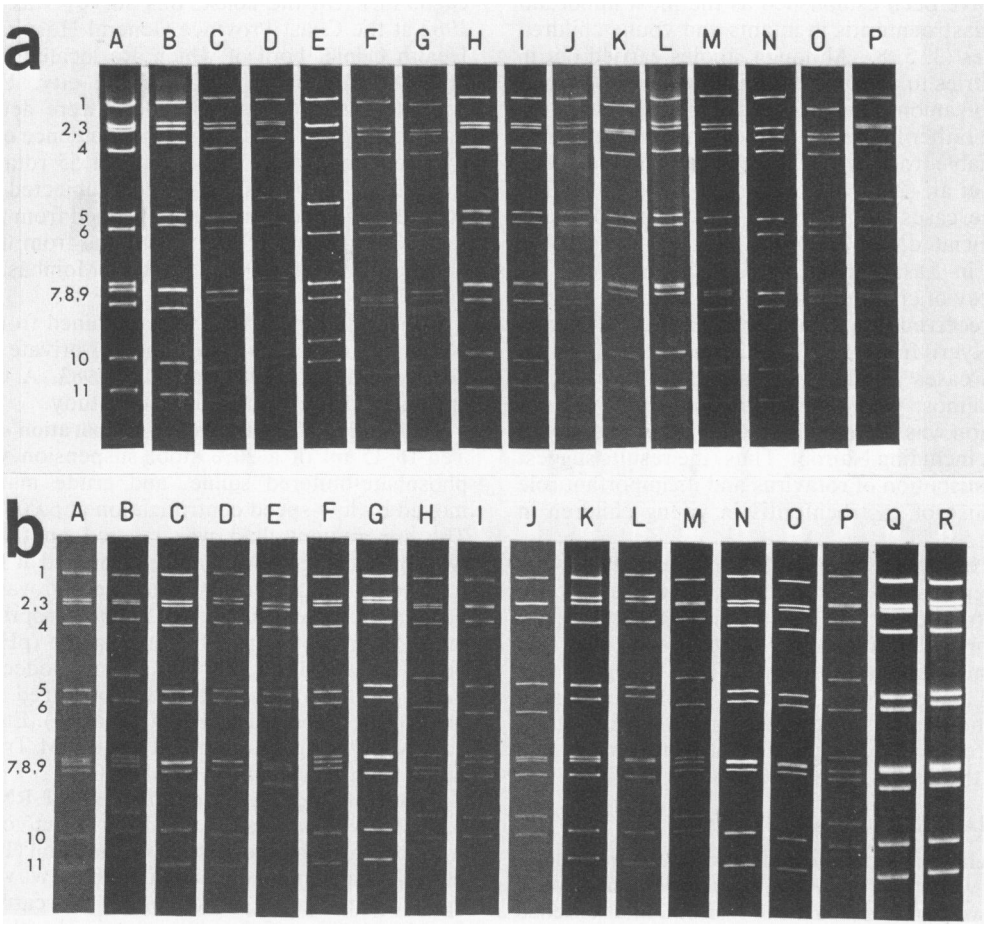

FIG. 1. Electrophoretic migration patterns of the genomic RNA of rotaviruses obtained from the coast (a) and from Nairobi (b). Independent alphabetical designation was employed for the classification of electropherotypes detected in each district. Migration was from top to bottom, and the RNA segments are numbered from the top. Since the figure was made from portions of photographs taken of polyacrylamide gels in different experiments, direct comparison of each electropherotype in this figure is not possible. Nevertheless, the presence of short types in coastal areas (D, E, and P) and in Nairobi (B, H, I, J, M, and P) are apparent.

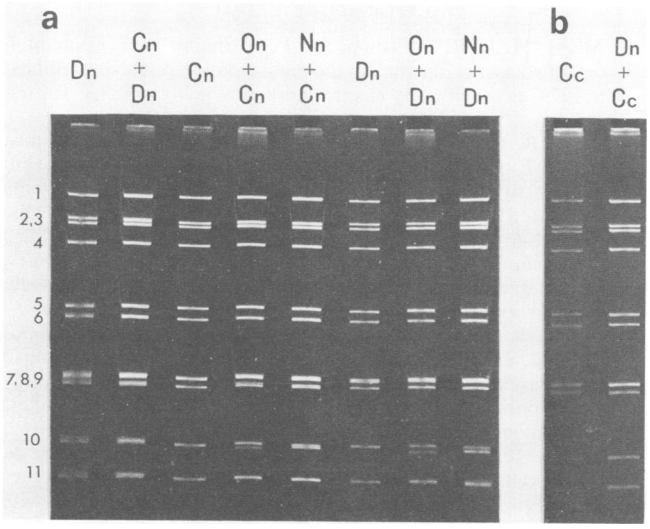

FIG. 2. Coelectrophoretic migration patterns of the genomic RNA of four rotaviruses (types Cn, Dn, On, and Nn) obtained from Nairobi (a), and two rotaviruses (types Cc and Dn) obtained either from the coast or from Nairobi (b). Migration was from top to bottom, and the RNA segments are numbered from the top.

sis by coelectrophoresis was necessary to determine the precise differences in electrophoretic mobility between corresponding RNA segments. The results obtained from three groups, each of which included four to five representative strains with similar electrophoretic patterns, are shown in Table 1. Of these, actual coelectrophoretic patterns of strains C, D, O, and N from Nairobi, designated Cn, Dn, On, and Nn, are shown in Fig. 2a. Although strains On and Nn showed common segment variations against strains Cn (segments 9 and 10) and Dn (segment 10), the coelectrophoretic migration pattern examined in the same gel clearly demonstrated that the former two strains had different degrees of electrophoretic mobility against segments 9 and 10 of strain Cn and segment 10 of strain Dn. Thus, all of these four strains were found to be different. In this series of experiments, strain C from the coast, designated Cc, and strain Dn were identified as being the same when examined by coelectrophoresis (Fig. 2b). The short strain P from the coast and strain H from Nairobi looked similar; however, owing to a lack of enough specimens, identification of these viruses could not be made.

Molecular epidemiology of rotavirus. The monthly occurrence of rotaviruses assigned to each electropherotype on the coast is shown in Table 2. Until the end of 1982, strains B, C, and H were more frequently detected than others. It is noteworthy that all three viruses obtained from the Waa Dispensary in October and November belonged to strain B. Subsequently, since early 1983, when the new electropherotypes M and N were first detected, striking changes in the virus populations prevailing in these areas have been observed. Of these two electropherotypes, strain N showed considerable segment variations as compared with those of previously predominant strains B (segments 1, 2, 3, 4, 5, 6, 7, 9, and 10) and C (segments 2, 5, 6, 7, 9, and 10). However, relatively little segment variation of another newly occurring strain, M, against strains B (segments 1, 4, and 6) and C (segments 6 and 9) made the interpretation of the role of such segment variations in causing changes in virus populations difficult.

The results also suggested that the electropherotypes M and N were introduced into the Kilifi district from the Mombasa urban area, where these strains had initially been detected (see below).

The determination of predominant electropherotypes in Nairobi was not successful owing to the small number of specimens relative to the wide range of variation, although some viruses, such as D and G, had been detected on several occasions during the period of this survey.

DISCUSSION

The present analysis of electrophoretic migration patterns of the genomic RNA of rotaviruses from two major districts of Kenya demonstrated the existence of various strains of the virus in a manner essentially similar to those in other countries (4, 14, 15).

From July 1982 to May 1983, 16 different electropherotypes were found in specimens obtained from the coast areas. In contrast, 18 strains, including six short ones, were recognized in Nairobi from January 1982 to May 1983. However, the number of virus specimens from this district subjected to analysis was too small to determine the predominant electropherotypes. This also suggested that the viruses detected in this study of Nairobi might be much smaller in number than the actual strains present.

TABLE 2. Number of rotaviruses assigned to each electropherotype by month of detection in coastal areas

Yr and mo	Electropherotype[a]															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1982																
July	1	4	2	1												
August			2		1	1										
September			1				2	3	1							
October		3 (1W)	1			1				1	1	1				
November		2 (2W)	1					1								
December																
1983																
January		1	1					1					1			
February		2											3 (1K)	7	1 (1K)	
March		1						1					7 (3K)	3 (3K)		1
April													6 (4K)	1		
May													2			

[a] Number and letter in parentheses indicate the number of viruses obtained from either the Waa dispensary (W) or the Kilifi District Hospital, Kilifi (K), among viruses assigned monthly to each type.

Among electropherotypes detected in the two districts, only one set of strains was found to be identical. Strain C from the coast was one of the predominant viruses in the area, and strain D from Nairobi was also recovered from three patients on different occasions. The presence of such a common strain may be a reflection of frequent exchange of people between these two geographically remote areas (480 km). In general, however, the prevalence of mutually unrelated strains in the two areas was similar to the observations of Schnagl et al. in Australia (15) and indicates the endemic nature of rotavirus infection in heavily populated urban areas.

In Mombasa, the appearance of newly predominant strains M and N was observed in early 1983, suggesting the presence of sequential changes (14, 15) in the rotavirus population circulating in the community. In addition, we could observe an association of the emergence of these new strains with the increased incidence of rotavirus gastroenteritis in Kilifi (population, 5,800), which is about 50 km north of Mombasa. Our survey in the Kilifi District Hospital started in November 1982, and the incidence of rotavirus infection among hospitalized children was rather low (11% on the average) until January 1983. From February 1983, however, a sharp increase in rotavirus infections was observed, with a maximum incidence of 47% occurring in March. The electropherotypes detected from the patients were M and N, which had initially been detected in Mombasa in January and February 1983, respectively. The results suggest that the epidemics of rotavirus in this area were due to viruses which were introduced from Mombasa and that they may represent a mode of virus spreading to a neighboring district where the population is too low to maintain endemism of the rotavirus (1, 11).

It was rather surprising to identify so many different electropherotypes from both districts in such a relatively short period of time in comparison with other reports (14, 15). It may be possible that the detection of these strains reflects a particular condition of children, in which rotavirus gastroenteritis tends to be more severe than usual even after infection with strains of lower pathogenecities (14). However, it should also be noted that rotavirus gastroenteritis was detected during any given month within this survey. Thus, the presence of various electropherotypes appears to have a close association with this particular nature of rotavirus epidemics in Kenya. A large pool of susceptible populations would allow a persistence of each type and thus favor an emergence of genetically reassorted viruses among them (4, 14, 15). Recent observations in different settings revealed the relatively important role of climatic factors for the epidemics of rotavirus infection (8, 9, 12). In addition, the present results clearly indicate that characterization of rotaviruses is important to elucidate an epidemiological nature of the infection in tropical areas in more detail.

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