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Four-Year Study of Rotavirus Electropherotypes from Cases of Infantile Diarrhea in Rome

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Rotavirus infections were detected in 210 of 675 children with acute diarrhea admitted to a major pediatric hospital in Rome from January 1982 through December 1985. Most of the patients with rotavirus infections were admitted during the winter season in both 1982 and 1985, whereas during the two intermediate years, cases occurred in all months. Among 84 rotavirus samples examined, 14 different electropherotypes were recognized, 2 of which largely predominated over the others. The two electropherotypes were particularly frequent in the 2 epidemic years, altogether accounting for 70.2% of the samples typed, and circulated in distinct periods. None of the viruses showed a short pattern of electrophoretic migration of the genome, indicating a minor involvement of subgroup I rotaviruses in hospitalization-requiring diarrheas occurring in the area surveyed.

Human rotaviruses (HRV) constitute a heterogeneous group of viruses showing different antigenic specificities and a high extent of genetic diversity. The identification of virus strains through serological methods, by which at least two antigenically different subgroups and six serotypes of HRV can be distinguished (3, 9, 15, 24, 28, 41), is hampered by the difficulties encountered in growing human rotavirus in cell cultures and by the close antigenic relatedness of various strains. As an alternative approach, analysis of the virus genome by polyacrylamide gel electrophoresis (PAGE) has proven to be valuable for the differentiation of field strains of HRV, especially in large-scale studies, and has been adopted by many laboratories (1, 7, 12, 14, 16, 27, 32, 35). Infected people usually shed large amounts of rotavirus with their feces, and in many cases, enough viral genomic RNA for analysis can be directly extracted from minimal volumes of stools (12, 29, 40), thus circumventing the need for prior virus isolation and purification. The rotavirus genome consists of 11 segments of double-stranded RNA which can be resolved as separate bands by electrophoresis (23). Variations in the electrophoretic mobility of one or more segments allow different HRV strains to be assigned to distinct electropherotypes.

In the present paper, we report the results of a 4-year study of the occurrence of HRV infections among children with acute diarrhea in the area of Rome. The diffusion of different rotaviral strains was investigated by PAGE of viral genomic RNA for strain identification.

MATERIALS AND METHODS

Patients. Fecal samples were obtained from children aged 0 to 3 years admitted to the Bambino Gesú Hospital in Rome from January 1982 to December 1985. All patients hospitalized with a diagnosis of acute diarrhea within 5 days from the onset of symptoms were included in the study; all patients had signs of dehydration and had passed three or more loose or watery stools daily for at least 1 day. For each patient, a single stool sample was collected during the first 24 h of admission.

HRV detection. Suspensions (20%) of feces in phosphate-buffered saline (pH 7.2) were extracted with trichlorotrifluoroethane and tested for rotavirus group-specific antigen in an enzyme-linked immunosorbent assay (Rotazyme; Abbott Laboratories, North Chicago, Ill.) according to the instructions of the manufacturer. Electron microscopic examination of samples was performed as previously described (31).

Rotaviral RNA extraction. Rotavirus-positive stool extracts, prepared as described above, were diluted twofold with RNA extraction buffer (0.01 M Tris, 0.1 M NaCl, 0.001 M EDTA, 1% sodium dodecyl sulfate, pH 7.5) and were deproteinized with a mixture of phenol, chloroform, and isoamyl alcohol. RNA was precipitated from the aqueous phase by adding 2 volumes of cold ethanol in the presence of 0.3 M sodium acetate and incubating overnight at −20°C. After centrifugation at 8,000 × *g* for 30 min, the pelleted RNA was dissolved in Laemmli sample buffer (26) containing 0.003% bromophenol blue and 2.5% Ficoll 400 (Pharmacia Fine Chemicals, Piscataway, N.J.).

Electrophoresis of rotaviral RNA. PAGE was carried out by the method of Laemmli (26) in slab gels of 10% acrylamide and 0.35% bisacrylamide, with a 3.5% acrylamide stacking gel. Runs were performed for 20 h at a constant voltage of 180 V. Gels were silver stained by the method of Herring et al. (18).

Statistics. Comparisons between the distribution of rotavirus infections by season in the different years were performed by the χ^2 test.

RESULTS

Stools were collected from 675 children with diarrhea admitted to the Bambino Gesú Hospital in Rome during the period from January 1982 to December 1985. On the whole, rotavirus was found in the feces of 210 patients (31.1%); infection rates ranged from 21.6% in 1983 to 41.2% in 1985, being 29.6 and 37.6% in 1982 and 1984, respectively.

In 1982, most of the patients with rotavirus diarrhea (58 of 63, 92.1%) were admitted during the first half of the year (Fig. 1). In contrast, in both 1983 and 1984, rotavirus-

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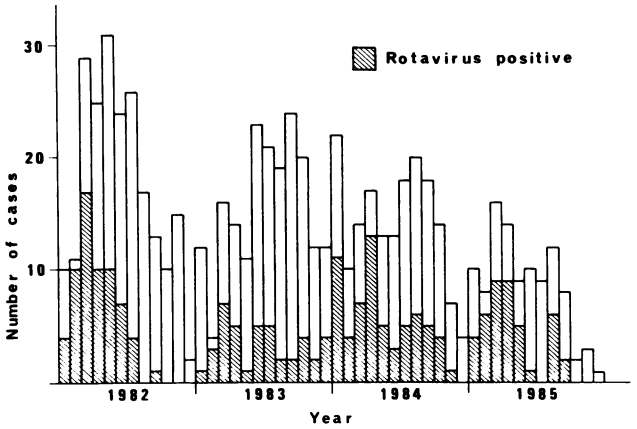

FIG. 1. Rotavirus infections in children with diarrhea admitted to the Bambino Gesú Hospital (Rome, Italy) from January 1982 through December 1985.

positive cases occurred in all months and were distributed through a series of minor epidemic waves. In fact, the distribution of cases of rotavirus diarrhea by season (Table 1) during 1982 proved to be very different from those observed in 1983 ($\chi^2 = 23.1$, $P < 0.001$) and 1984 ($\chi^2 = 11.3$, $P < 0.01$). However, it did not differ from the distribution of cases during 1985 ($\chi^2 = 1.3$, $P > 0.1$). Indeed, during the last year of the survey, the frequency of rotavirus infections increased and reached a major peak in winter and spring, followed by a relatively smaller cluster of cases in August and September.

Rotavirus double-stranded RNA was extracted from the feces of infected children and analyzed by PAGE. An amount of RNA sufficient to perform comparisons between strains, including coelectrophoretic runs of closely similar electropherotypes, was recovered from 84 samples. Among the typed rotavirus samples, 14 electropherotypes were distinguished and were designated A through N (Fig. 2). As compared with the mobility of band 11 of bovine rotavirus (not shown), all samples tested exhibited the long pattern of migration that is typical of subgroup II rotaviruses. In one case, more than 11 segments of rotaviral RNA were extracted from the same stool sample, indicating the occurrence of infection with different rotavirus strains (not shown). Electropherotypes A and I were by far more common than any of the other 12 rotavirus electropherotypes detected, altogether accounting for 59 (70.2%) of the 84 samples studied.

The quarterly distribution of rotavirus electropherotypes is reported in Table 2. Electropherotype A accounted for 9 of 10 samples examined in 1982 and was also found to be the predominant type in 1983 (39.1% of samples tested), during which nine further electropherotypes were encountered. In

TABLE 1. Distribution of cases of rotavirus diarrhea at the Bambino Gesú Hospital in Rome by season, 1982 through 1985

Season	No. of cases (%) in:			
	1982	1983	1984	1985
Winter	26 (41.3)	9 (23.1)	22 (33.3)	16 (38.1)
Spring	30 (47.6)	11 (28.2)	22 (33.3)	18 (42.9)
Summer	7 (11.1)	10 (25.6)	15 (27.7)	8 (19.0)
Autumn		9 (23.1)	7 (10.6)	

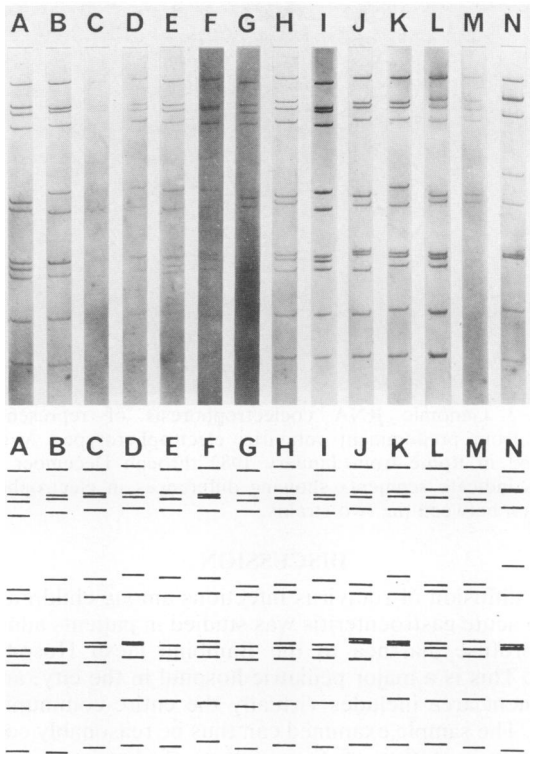

FIG. 2. Genomic RNA profiles (upper panel) and schematic representation (lower panel) of representative rotavirus strains from electropherotypes identified in Rome from January 1982 through December 1985.

1984, six electropherotypes were revealed, two of which were newly introduced; interestingly, no sample belonged to the formerly established electropherotype A, and most of the samples (65.4%) were assigned to electropherotype I, occurring for the first time in December 1983. Of the 25 virus samples characterized in 1985, 23 (92%) were found to belong to electropherotype I. When compared by coelectrophoresis (Fig. 3), electropherotypes A and I showed different mobilities for 7 of the 11 genome segments.

TABLE 2. Distribution of HRV electropherotypes by quarter in Rome, 1982 through 1985

Electropherotype	No. of cases with electropherotype during quarter of:												
	1982, 2	1983				1984				1985			
		1	2	3	4	1	2	3	4	1	2	3
A	9	1	5	2	1								
B	1	1				2							
C		1	1				1						
D		1											
E			1										
F				1	2								
G				1									
H				1	1	1	1						
I					1	11	2	4		10	11	2	
J					2								
K						1	1		1				
L								1					
M											1		
N												1	

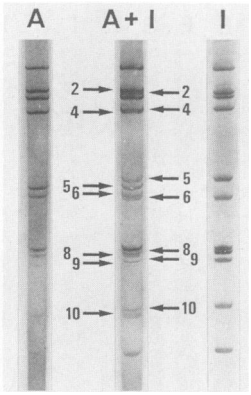

FIG. 3. Genomic RNA coelectrophoresis of representative strains from predominant rotavirus electropherotypes A and I identified in Rome from January 1982 through December 1985. Arrows indicate segments showing differences in electrophoretic migration between the two strains.

DISCUSSION

The diffusion of rotavirus infections among children with severe acute gastroenteritis was studied in patients admitted with profuse diarrhea to the Bambino Gesú Hospital in Rome. This is a major pediatric hospital in the city, and its catchment area includes virtually the entire community of Rome. The sample examined can thus be reasonably considered representative of the portion of diarrhea episodes occurring in the area that are severe enough to require hospitalization.

An unusual finding in this investigation was the considerable seasonal variation in the rate of hospitalization because of rotavirus diarrhea over the years. The typical epidemiological pattern of infection described for countries with a temperate climate (4, 25) was observed in Rome only in 1982, when virtually all patients with rotavirus infections were clustered in a single peak across the coldest months. The two following years, however, were characterized by the presence of cases in all months, suggesting an endemic persistence of the virus in the population. Finally, a partial return of the winter-associated pattern of disease occurred in 1985, when, however, a second epidemic peak of infection was detected between August and September. Such a succession of different epidemiologic pictures appears to contrast with the findings of other investigators, whose long-term surveys recorded a strict precision of the annual waves of rotavirus infections (4, 25). In a preliminary analysis of the epidemiological data from this sample up to December 1984 (11), we excluded the possibility that climatic factors might have modified the spread of rotavirus through the population. In fact, no relevant change was observed in the annual trend of either temperature or relative humidity between years. Rather, we hypothesized that the epidemic diffusion of rotavirus in the first half of 1982 might have evoked a broad herd immunity. Thereafter, the level of susceptibility in the population might have remained so low as to hinder the onset of new epidemic outbreaks of disease in 1983 and 1984. Consistently, we found that the median ages of patients with rotavirus infection were 17, 10, and 11.5 months, respectively, for 1982, 1983, and 1984, as against 9, 9, and 10 months, respectively, in children negative for rotavirus. In 1985, patients with and without rotavirus infection had median ages of 13 and 10 months, respectively.

The results of the genomic characterization of viral strains allow further considerations to be made. During the 4 years of the study, we detected 14 different rotavirus electropherotypes, among which electropherotypes A and I were by far more frequent than the others. Similar findings were previously reported by several authors (10, 12, 14, 32, 33, 35, 36). Interestingly, the two predominant viral electropherotypes revealed in this study were particularly common during the 2 epidemic years, electropherotype A accounting for 90% of samples typed in 1982 and electropherotype I accounting for 92% of the samples in 1985. Because of the lower proportion of virus samples typed in 1982 as compared with the other years, it cannot be excluded that strains from other less-represented electropherotypes circulated that year in addition to the virus strain represented by electropherotype B. However, this does not appear to invalidate the conclusion that viral electropherotype A largely predominated during the epidemics of disease in 1982.

The disappearance at the end of 1983 of electropherotype A, which had formerly predominated, seems consistent with the hypothesis suggested by our epidemiologic data, according to which a broad herd immunity had been established in the population in that period. Indeed, during 1983, as many as eight different minor electropherotypes besides electropherotype A were found, suggesting that the virus strain represented by this electropherotype was under increased selective pressure. In this view, the spread of the strain represented by the new predominant electropherotype I during 1984 might have been favored by the possible existence of marked antigenic diversity between this strain and that represented by electropherotype A. Accordingly, Uhnoo and Svensson (38) have recently described the succession of long and short electropherotypes, suggesting that this may reflect a change in the immunity of the population at risk. However, except for the assumed association of the short electropherotype and serotype 2 rotaviruses (22), it has been shown that rotavirus strains belonging to different electropherotypes do not necessarily exhibit different serotype specificities (2), thus implying that electropherotyping per se is not sufficient to establish the antigenic correlations between strains. On the other hand, several lines of evidence suggest that the relationship between in vitro neutralization, and hence serotyping antibodies, and in vivo protection is not as obvious. Immunization studies carried out on human volunteers indicate that attenuated animal rotavirus vaccines can also afford protection against serotypically unrelated rotaviruses (39). Heterotypic serological responses have also been observed during natural infection (6, 8). In addition, a number of studies (21, 30, 34, 37) have revealed that both VP7, the major surface antigen of rotavirus, and the outer protein VP3 elicit the production of neutralizing antibodies and that some of these are broadly cross-reactive with different virus serotypes. The presence of multiple antigenic determinants in both VP3 and VP7 and the independent segregation of the genetic information for these proteins (20, 21, 34) appear to suggest that conventional serotyping may not entirely account for the actual antigenic differences among viral strains. Because of its high sensitivity in distinguishing individual rotavirus strains, genome fingerprinting may thus allow relevant epidemiological observations, which serotyping alone could dismiss. Whatever the serotypes of the virus strains they represent may be, it is clear that electropherotypes A and I in our study correspond to two actually distinct rotavirus strains; in fact, they shared only five genome segments with identical electrophoretic mobilities. Interestingly, segments 4 and 9, which carry the genes for VP3 and VP7, respectively, migrated differently in the two strains.

Variation in virulence has been recently shown for animal rotaviruses (5) and is also thought to occur among human strains (13). We cannot rule out the possibility that the selection of strains represented by electropherotypes A and I in our population may have been at least partially favored by a more pronounced virulence of these strains with respect to all the others. However, it is unlikely that replacement of the strain represented by electropherotype A with the strain represented by electropherotype I as the predominant strain occurred for this same reason, since the decline of the former electropherotype was already completed at the end of 1983 when electropherotype I emerged. On the other hand, a preliminary analysis of clinical signs (data not shown) revealed no appreciable differences between rotavirus-infected patients by year of admission or type of infecting virus.

It is noteworthy that despite the circulation of many different rotavirus strains, in no case did we find an infection with a short-pattern rotavirus. Among human virus strains, the presence of the short electropherotype is strongly associated with subgroup I and serotype 2 specificities (22); rotaviruses with these characteristics are considered as forming a closely related family distinct, from an evolutionary point of view, from subgroup II viruses (17, 19). Even though only 40% of our samples could be typed by electrophoresis, our results suggest a minor involvement, if any, of such viruses in cases of diarrhea requiring hospitalization in our country.

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