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Variation in Human Rotavirus Electropherotypes Occurring Between Rotavirus Gastroenteritis Epidemics in Central Australia

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The changes in human rotavirus electropherotypes, occurring during a period including two rotavirus gastroenteritis epidemics in 1976 and 1979 in relatively remote Central Australia, were determined by polyacrylamide gel electrophoretic analysis of the rotavirus genome ribonucleic acid. A number of different electropherotypes were present during each of the epidemics, although a single type was predominant in each one. The predominant electropherotype of the first epidemic persisted in the area for approximately 2 years afterwards. Apart from this electropherotype, only three others were recognized in the 3 years between the two epidemics. One of these, first seen 1 year before the second epidemic, bore a very close similarity to the predominant type of the second epidemic. Altogether, 12 different electropherotypes were recognized during the period of the survey. No type common to both areas was found when rotavirus electropherotypes recognized in Central Australia were compared with those detected in a 1973-to-1979 survey in Melbourne, Australia.

Human rotavirus has been recognized for a number of years as a major cause of infantile diarrhea (7), and although many studies on its incidence and distribution have been carried out, few seroepidemiological surveys have been undertaken. This is understandable in view of the difficulties presented by the close antigenic relatedness of the various animal rotaviruses to human rotavirus and the problems associated with growing human rotavirus strains in cell culture (7, 23). A further problem is that although the existence of different human rotavirus serotypes has been recognized for some time (4, 20, 24, 25), uncertainty still exists as to the number.

It has been known since 1976 that variations in the molecular weights of genome segments exist among different isolates of rotavirus from a single species, and the implications of this for epidemiological studies have been recognized in recent years (1-3, 9, 14, 15, 21, 22). However, epidemiological interpretation of these rotavirus genome variations has generally been somewhat restricted because of the small numbers of specimens or the relatively limited time periods that were investigated, and it was not until the comprehensive survey of Rodger et al. (13) that the extent of the usefulness of this approach to rotavirus epidemiology was really underlined. Rodger et al. (13), using stool specimens obtained from hospitals serving mainly the urban area of Melbourne, Australia, reported an apparent succession of at least 19 human rotavirus electropherotypes in that city between 1973 and 1979. Before their survey, the detection of only two or three human rotavirus electropherotypes had been reported (1-3, 9).

It is, of course, true that differences in electrophoretic patterns of rotavirus genomes do not necessarily reflect changes in serotypes, but gel electrophoretic analysis of virus genomes for strain identification has already been applied in retrospective studies on known serotypes of influenza virus (6, 11), orbiviruses (5), and reoviruses (8, 12).

In this survey, carried out between 1976 and 1979, we looked at changes in human rotavirus electropherotypes occurring between rotavirus gastroenteritis epidemics in relatively remote Central Australia in the only major city of the area, Alice Springs, and in settlements within a 500-km radius of Alice Springs. Two major and distinct epidemics of infantile diarrhea due to rotavirus occurred in the area during the time of the survey, in June and July 1976 (17) and in August and September 1979 (Schnagl and Morey, unpublished data). Although Rodger et al. (13) in their survey observed an increased winter incidence of rotavirus, they were not able to study rotavirus electropherotype variations between completely distinct epidemics.

As the electropherotypes recognized in this

survey were also compared with those of the Melbourne survey of Rodger et al. (13), the results provide not only some insight into the variation and persistence of rotavirus electropherotypes between epidemics but also into the variation in electropherotypes between geographically widely separated areas within the same country.

MATERIALS AND METHODS

Virus specimens. Stool specimens were obtained from Aboriginal and European children and adults with diarrhea who had been admitted to Alice Springs hospital between 1976 and 1979. Specimens were obtained continuously during the whole of 1977 and 1978 but only during the actual rotavirus gastroenteritis epidemics of 1976 (June and July) and 1979 (August and September). Also, stool specimens were obtained from some children and adults with diarrhea who had not been admitted to the hospital. Ages ranged from 2 months to 80 years.

The hospital in Alice Springs (population, 15,000) serves an area of Central Australia covering several hundred thousand square kilometers. Apart from Alice Springs, the area includes only a number of fairly small remote settlements. The total population served by the hospital numbers approximately 20,000. Although Alice Springs is about 1,800 km by air from Melbourne (population, 2,500,000), there is quite a deal of tourist traffic between the two cities, approximately 25,000 people per year, as well as between Alice Springs and other areas of Australia.

For electron microscopic examination for rotavirus, after homogenization the specimens were partially purified by low-speed centrifugation, and then the virus was pelleted by high-speed centrifugation as outlined previously (16). Of the 1,034 specimens examined, 162 were positive, and 872 were negative for rotavirus. Of the 162 rotavirus-positive specimens, 154 of which came from children less than 2 years of age, and 3 of which came from adults, only 51 yielded sufficient viral ribonucleic acid (RNA) for visualization on polyacrylamide gels. Of these specimens, 49 came from children less than 2 years of age, and two came from older children. The percentage of specimens that was possible to analyze for rotavirus RNA was approximately constant over the whole period of the survey.

Deproteinization and electrophoresis of viral RNA. Partially purified virus as prepared for electron microscopy was further purified by fluorocarbon extraction, disrupted with sodium dodecyl sulfate, and then deproteinized with phenol or a combination of phenol, chloroform, and isoamyl alcohol as described by Rodger and Holmes (14). Pelleted RNA was dissolved in Laemmli sample buffer (10) modified to contain double the amount of glycerol.

Electrophoresis of deproteinized RNA was carried out in 10% polyacrylamide slab gels with the discontinuous buffer system, described by Laemmli (10) and used for RNA by Ramig et al. (12), and continued for 4.5 h at room temperature as described previously by Rodger and Holmes (14). Gels were stained overnight with ethidium bromide at a concentration of 5 μg/ml before photography. Comparisons of different human rotavirus stains were made by mixing and then coelectrophoresing the RNAs.

RESULTS

After cross-comparison, in most cases by coelectrophoresis, 12 different human rotavirus electropherotypes were recognized in Central Australia from 1976 to 1979. Coelectrophoresis was not carried out between very obviously different types as electrophoresis on the same gel was considered sufficient to establish the differences between them. The 12 electropherotypes were labeled A to L, and the differences in the relative mobilities of their genome segments are shown on the one gel in Fig. 1. These electropherotypes are not the same as those reported by Rodger et al. (13); the type of nomenclature adopted by these authors has been used here only for convenience. Sufficient quantities of electropherotypes C (1978), I, and L were not available for the final gel, but so as to give a better overall comparison, profiles for these types were drawn in (Fig. 1).

The electropherotypes recognized, including the apparently predominant one, are listed in Table 1 by the year of detection. It should be noted, however, that all of the specimens in this study were obtained from individuals with symptoms of diarrhea which, in the vast majority of cases, were severe enough for them to have been admitted to the hospital. Electropherotypes recognized in surveys such as this may therefore not necessarily present a complete picture of the real situation, as electropherotypes in very mild or asymptomatic infections would not have been accounted for.

From the extensive rotavirus gastroenteritis epidemic which occurred during the winter of 1976, three different electropherotypes were recognized, with type C being predominant (Fig. 1 and Table 1). Electropherotype C was found in stool specimens from children from four different settlements as well as in the stool of a child from Alice Springs. Electropherotype A (Fig. 1) is shown with band 4 apparently split. This was concluded, since another 1976 electropherotype was found to be identical to that shown as type A in Fig. 1, except that only one band was present in the region of band 4. Both of these electropherotypes were considered as being type A in this survey. Sekiguchi and Koide (19) showed a splitting of bands after polyacrylamide gel electrophoresis of reovirus type 3 genome RNA. However, another explanation of the double band could be the occurrence of a dual infection in this child.

In 1977, only two different electropherotypes were recognized, and one of these was identical

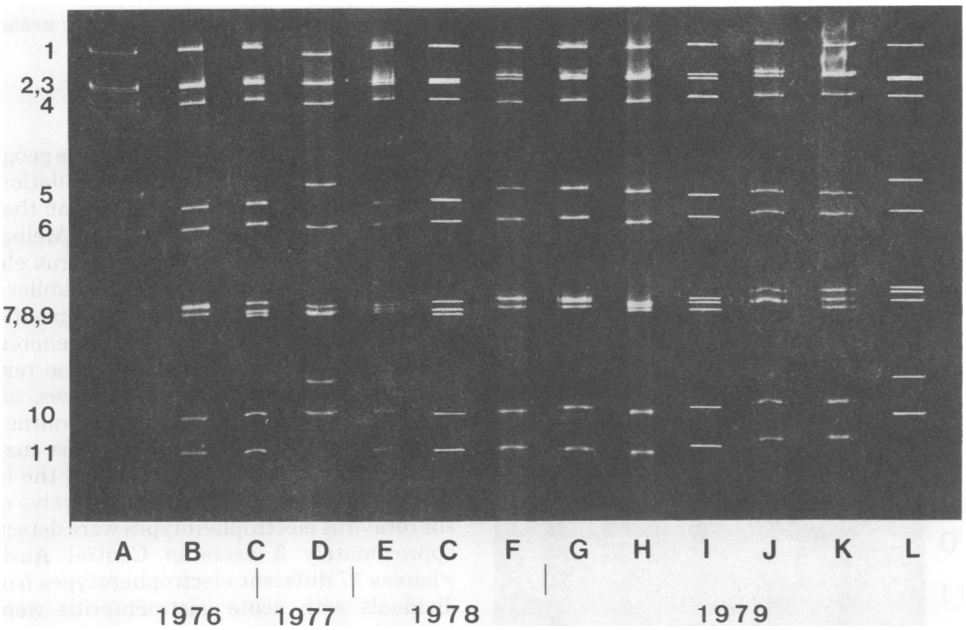

FIG. 1. *Comparison of electrophoretic patterns of human rotavirus electropherotypes A to L recognized in Central Australia in 1976 to 1979. Migration was from top to bottom, and genome segments are numbered in descending order of size. The bands shown for electropherotype C from 1978, electropherotype I, and electropherotype L are accurate diagrammatic representations of the actual RNA profiles (see the text).*

TABLE 1. *Rotavirus electropherotypes recognized in Central Australia*

Yr	Total no. of samples positive for rotavirus by electron microscopy	No. of samples with sufficient rotavirus for electrophoresis	Electropherotypes recognized	Predominant electropherotype (no. of samples with predominant type)
1976 (During epidemic, June–July)	50	10	A, B, C	C (6)
1977 (Whole yr)	38	15	D, C	D (9)
1978 (Whole yr)	25	8	C, E, F	C (5)
1979 (During epidemic, August–September)	49	18	G, H, I, J, K, L	G (10)

to the apparently predominant type C of the 1976 epidemic (Fig. 1 and Table 1). Coelectrophoresis of type C from 1976 and type C from 1977 is shown in Fig. 2. It is interesting to note that type C from 1977 was found only from October to December of that year and was apparently responsible for a small but fairly extensive rotavirus gastroenteritis outbreak in November (spring) 1977 (18). The ages of the children involved in this outbreak ranged from 3 to 18 months, with the majority being less than 12 months, i.e., the majority of these children were born after the 1976 type C outbreak. The other electropherotype (type D), which appeared to be quite different from any of the previous types and was of the "short" variety, was detected only from January to August. Both electropherotypes were found in stools of children from Alice Springs and a number of other different settlements.

Very little gastroenteritis attributable to rotavirus was found in 1978, although three different human rotavirus electropherotypes were recognized (Fig. 1). One of these was again called type C as it proved to be identical with the type C of 1976 and 1977. Coelectrophoresis of type C from 1977 and type C from 1978 is shown in Fig. 2. Although type C appeared to have been the predominant electropherotype of 1978 (Table 1) it was only found in the first half of the year. Electropherotype C was detected in children from three different settlements as well as in children from Alice Springs.

Six different electropherotypes were recog-

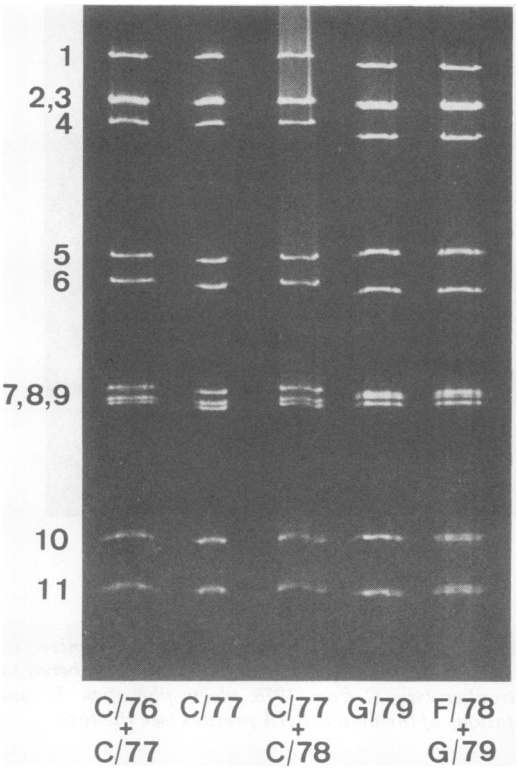

FIG. 2. *Coelectrophoresis of rotavirus electropherotypes C from 1976, 1977, and 1978 (C/76, C/77, and C/78, respectively), F from 1978 (F/78), and G from 1979 (G/79). Migration was from top to bottom, and genome segments are numbered in descending order of size.*

nized during the rotavirus gastroenteritis epidemic of August and September (winter-spring) 1979, with type G being the predominant one (Fig. 1 and Table 1). Five of the electropherotypes were detected in Alice Springs. Electropherotype G was found in stools from children and adults from Alice Springs and in stools from children from five other settlements. It is interesting to note the very close similarity between type F, seen in May 1978 and type G, the predominant electropherotype of the 1979 epidemic. Coelectrophoresis of types F and G is shown in Fig. 2. It is also interesting to note what appears to be the relatively rare reappearance of a short electropherotype (type L), although type L is different from the other short electropherotype D of 1977. Type L was recognized in only one instance.

The 12 electropherotypes recognized in Central Australia in this survey were compared with the 19 types found by Rodger et al. (13) in their 1973-to-1979 Melbourne survey, where considered necessary, by coelectrophoresis, but no electropherotype common to both areas was found.

DISCUSSION

The area covered in this survey was geographically quite different, and the population was smaller and much more scattered than that surveyed by Rodger et al. (13) in urban Melbourne, but the results from the two rotavirus electropherotype surveys are remarkably similar. This lends further support to the concept of using electropherotyping in rotavirus epidemiology.

The Central Australian survey also revealed a changing population of rotaviruses, as was found by Rodger et al. (13) in Melbourne, and, as suggested by these authors, this may, by analogy with influenza virus, reflect the occurrence of antigenic drift and shift. Twelve different rotavirus electropherotypes were detected in approximately 3 years in Central Australia, whereas 17 different electropherotypes from individuals with acute gastroenteritis were detected in approximately 7 years in Melbourne. The slightly greater rate of detection of different electropherotypes in a smaller population may be a reflection of the two epidemics that occurred during this survey, together with the sampling of a more scattered population in a larger geographical area.

It would appear that during epidemics a somewhat larger number of different rotavirus electropherotypes may emerge, with one type being predominant. The finding of predominant and less common human rotavirus electropherotypes in a community is in agreement with not only the findings of Rodger et al. (13) but also those of Espejo et al. in Mexico City (2, 3) and Croxson and Bellamy in Auckland, New Zealand (1).

Rodger et al. (13) found in Melbourne that the predominant electropherotype of the large winter rotavirus gastroenteritis outbreaks first appeared in the community in the previous spring, but only in relatively few individuals. Some confirmation of this may have been found in the Central Australian survey when the very close similarity between the predominant electropherotype G of the 1979 rotavirus gastroenteritis epidemic and electropherotype F, detected more than 1 year before, is considered.

The apparent genetic stability of some human rotavirus genomes on passage in a community found by both Espejo et al. (2, 3) and Rodger et al. (13) is supported by our observations in Central Australia. Espejo et al. showed the persistence of rotavirus electropherotypes for 12 months, and Rodger et al. showed this for over 2 years in the case of the community in general and for 4 years in the case of neonates in hospital

wards. In Central Australia, we found that the predominant electropherotype C of the 1976 rotavirus epidemic persisted for approximately 2 years after the epidemic, and it will be of interest to see whether the major electropherotype G of the 1979 epidemic persists beyond 1979.

The absence of any close similarity between human rotavirus electropherotypes recognized in Central Australia and those found in Melbourne is a little surprising in view of the observed stability of the rotavirus genome in communities and the exchange of people that occurs between Alice Springs and Melbourne. Perhaps this exchange does not include enough infants to introduce sufficient virus for adequate spread, or possibly rotavirus genomes may change fairly rapidly when introduced into a new area, even if only slightly. Changes or new types could arise in dual infections as a result of reassortment between the genomes of the introduced virus and rotavirus types already circulating in the community, either human or animal. To determine whether the lack of close similarity between rotavirus electropherotypes from the two areas extends to a lack of antigenic similarity, adequate serotyping or establishment of the relationship between electropherotypes and serotypes will be required.

Overall, however, it has become clearer that, as is apparent with influenza virus (6) and reovirus (8), gel electrophoretic analysis of rotavirus genomes will provide more detailed epidemiological information than serotyping alone.

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