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Distribution of Both Rotavirus VP4 Genotypes and VP7 Serotypes among Hospitalized and Nonhospitalized Israeli Children

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Human rotaviruses belonging to genotype P9, of probable feline origin, which included both G3 and G1 serotypes, were detected in 3.8% of children shedding rotaviruses who attended sick fund clinics throughout Israel. None were detected in children admitted to hospitals because of severe diarrhea. In contrast, the relative prevalences of genotypes P8 and P4 were similar between the two groups.

The two outer capsid viral proteins VP7 and VP4 form the basis for the current classification of group A rotaviruses into G (VP7) and P (VP4) serotypes (4). To date, 14 G serotypes (1, 2, 4) and at least 18 P serotypes (19, 21) have been recognized among field isolates of animal and human rotaviruses. A distinction between P serotypes and P genotypes has been suggested (19). A P genotype includes group A rotaviruses having homology of 89% or greater in the deduced amino acid sequences of their VP4. A P serotype relates to group A rotaviruses shown to be related by one-way neutralization or cross-neutralization. Because of lack of data, not all P genotypes have been assigned a P serotype yet. Serotypes G1 to G4 (4) and genotypes P8 (Wa-like) and P-4 (DS-1-like) (9, 19) are widespread among human populations. Other human G serotypes and P genotypes, such as G8, G9, and G12 and such as P6 (M37-like) and P10 (69M-like), are less frequent. However, G serotypes 5, 6, and 10, which were thought to be exclusively animal viruses, have recently been recovered from children with diarrhea and from asymptomatic neonates (3, 8, 10). Certain P genotypes were also detected in both animals and humans. These included genotype P9 (AU-1-like), which specifies the AU-1 VP4 allele and was detected in both cats and humans (13). Another P genotype, P13 (HCR-3-like or Ro-1845-like), is common to all canine and certain feline rotaviruses and was also detected in human babies in Israel, the United States, and Brazil (14, 15, 22). Finally, genotype P11 (B-223-like), a bovine rotavirus, was detected in asymptomatic neonates in India (3, 6).

Several panels of G serotype-specific monoclonal antibodies are now available, but P serotype-specific monoclonal antibodies are harder to obtain. Consequently, investigators use cDNA probes made up from highly divergent and serotype-specific regions in gene 4 (20), the gene encoding the VP4 protein, and PCR techniques (7, 11, 18) in order to classify field isolates of rotaviruses into P genotypes.

We undertook this study in order to find out whether the distribution of rotavirus G serotypes and P genotypes in the community differed from that among hospitalized children with diarrhea.

Stool samples were collected between September 1991 and August 1994 from children 2 months to 4 years of age who were hospitalized in four major and two smaller hospitals in Israel because of severe diarrhea or who presented at 15 sick fund clinics throughout the country because of gastroenteritis. Of the 15 clinics, 13 serve the geographical regions where the hospitals are located.

The samples were checked for group A rotavirus antigen with a commercial enzyme-linked immunosorbent assay kit (Dakopatts, Denmark) and then were checked for G serotypes with a panel of G serotype-specific monoclonal antibodies (Silenus Co., Victoria, Australia) and for P genotypes by a PCR technique that involved extraction of RNA from the stool samples and two successive amplification steps and employed both consensus and nested primers. Nested primers flanking the P serotype divergent regions in gene 4 of the P8, P4, P6, P9, and P13 genotypes were included in the PCRs. The G serotyping was performed according to the instructions of the manufacturer. The P genotyping technique was previously described (11).

Of 359 rotavirus-containing samples from hospitalized children and 434 samples from nonhospitalized children, 309 (87%) and 320 (74%), respectively, could be classed into G serotypes and P genotypes (Tables 1 and 2). Genotype P9, with a frequency of 3.8%, was detected only in children presenting to clinics, whereas genotypes P8 and P4 were detected in both groups at approximately the same frequency. As expected, P8 isolates included G1, G3, and G4 viruses whereas P4 isolates were associated exclusively with serotype G2. Nine P9 isolates belonged to G3 and resembled similar viruses detected in Japan, Italy, and Venezuela (12, 20). Three samples contained genotype P9 viruses that registered with G1-specific monoclonal antibodies and thus resembled the K-8 strain isolated in Japan, which turned out to be a natural reassortant between an AU-1 feline-like rotavirus and a Wa-like human rotavirus (16).

TABLE 1. Distribution of rotavirus G serotypes and P genotypes among 309 hospitalized children

Serotype	No. (%) of strains belonging to indicated genotype[a]	
	P8	P4
G1	186 (60.2)	0 (0)
G2	0 (0)	21 (6.8)
G3	2 (0.7)	0 (0)
G4	100 (32.3)	0 (0)
Total	288 (93.2)	21 (6.8)

[a] No strain of any of the serotypes belonged to genotype P9.

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TABLE 2. Distribution of rotavirus G serotypes and P genotypes among 320 children who presented at sick fund clinics

Serotype	No. (%) of strains belonging to indicated genotype		
	P8	P4	P9
G1	188 (56.8)	0 (0)	3 (0.9)
G2	0 (0)	26 (8.1)	0 (0)
G3	6 (1.8)	0 (0)	9 (2.9)
G4	88 (27.5)	0 (0)	0 (0)
Total	282 (88.1)	26 (8.1)	12 (3.8)

No additional G serotypes and P genotypes were detected in the two study groups. Six samples revealed mixed infections by two G serotypes and were not included in this study.

These results indicate that rotaviruses carrying the AU-1 VP4 allele and belonging to genotype P9 are probably attenuated to some extent in the human host and cause sporadic, self-limited, and milder infections in babies, who consequently do not require hospitalization. This interpretation is also substantiated by the fact that during a 10-year period we did not find any animal-like strains among over 2,000 rotavirus-containing samples obtained from hospitalized infants. The genotype P9 viruses were detected in individual babies presenting at 9 of 15 sick fund clinics stretching from the far north of the country to the south. These viruses probably infected human babies as a consequence of a zoonotic infection by circulating feline rotaviruses or by reassortants formed between feline and human group A rotaviruses such as the K-8 strain.

It is known that experimental infections by heterologous rotaviruses are less severe and often asymptomatic (5). These data support the hypothesis that the genotype P9 viruses originated in a heterologous host (cats, in this case) rather than being genuine human pathogens.

The present study indicates that genotype P9 occurs only among human patients with milder infections. However, through reassortment, such viruses may incorporate viral genes that control both virulence and host range restriction in human rotaviruses and become invasive or more virulent, leading to persistent infections among humans or to infections requiring hospitalization. Gene 4 has been implicated as a determinant of virulence in rotaviruses (17). Our data are consistent with this determination.

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