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Diversity of Rotavirus Strains among Children with Acute Diarrhea in China: 1998-2000 Surveillance Study

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As part of a national rotavirus surveillance activity, we collected fecal specimens from 3,177 children with acute diarrhea in 10 regions of China between April 1998 and April 2000 and screened them for rotavirus. Rotavirus was detected in 41% (*n* = 1,305) of specimens, and in these, G1 was the predominant serotype (72.6%), followed by G3 (14.2%), G2 (12.1%), G4 (2.5%), G9 (0.9%), and G untypeable (0.7%). Among 327 G-typed strains tested for P genotype, 14 different P-G combinations were identified, with the globally common strains P[8]G1, P[4]G2, P[8]G3, and P[8]G4 representing 75.6% of all typed rotavirus strains. Among the uncommon strains, 11 were P[6]G9, and others included P[6]G1, P[6]G3, and five novel P-G combinations (P[9]G1, P[4]G1, P[4]G3, P[4]G4, and P[8]G2). Our results indicate that while the common rotavirus strains remain predominant, the diversity of strains is much greater than was previously recognized.

In 2000 and 2001, China introduced its first rotavirus vaccine for childhood immunization (19). The vaccine, which was derived from a lamb strain of rotavirus, was developed and produced by the Lanzhou Institute of Biological Products. It was targeted to prevent rotavirus, the most important cause of severe diarrhea in Chinese children, which infects virtually all children by 5 years of age (17). This and other vaccines under development hold the promise of substantially reducing the severe morbidity and mortality from this common childhood infection.

Rotavirus strains can be serotyped and genotyped on the basis of two outer capsid proteins that are the targets of neutralizing antibodies produced following natural infection, the glycoprotein VP7, which determines G serotypes, and the protease-sensitive protein VP4, which determines P types. Both proteins play important roles in protection (16, 21). Fourteen rotavirus G serotypes, including 10 that infect humans, and 21 P types, including 9 that infect humans, have been identified (6, 22). Serotyping and genotyping studies indicate that only five G-P combinations (P[8]G1, P[4]G2, P[8]G3, P[8]G4, and P[8]G9) are common worldwide (13), although large regional variation of G serotypes has been documented in some developing countries (14, 26). Natural infection and vaccine trials with Jennerian vaccines based upon animal strains of rotavirus suggest that homotypic protection may be greater than heterotypic protection to different G strains (2, 11). The Chinese vaccine is derived from a lamb strain of rotavirus characterized as P[12]G10, which shares neither G nor P type with the common human strains.

In anticipation of the introduction of rotavirus vaccines and in considering changes in strain diversity that might occur afterwards, we established a rotavirus strain surveillance program to monitor the diversity of rotavirus strains present in China over time. Herein, we report the results of a 2-year surveillance study of rotavirus strains collected from children with acute diarrhea in 10 regions of China.

Stool specimens were collected from children with acute diarrhea from April 1998 through April 2000 in 10 regions of China: Changchun, Jilin Province; Qinhuangdao, Hebei Province; Beijing; Zhengzhou, Henan Province; Hangzhou, Zhejiang Province; Fuzhou, Fujian Province; Guangzhou, Guangdong Province; Lanzhou, Gansu Province; Chengdu, Sichuan Province; and Kunming, Yunnan Province. Samples were stored at −20°C until tested for rotavirus at the Institute of Virology in Beijing by gel electrophoresis and enzyme immunoassay (EIA) (24, 28). EIAs for rotavirus G serotyping using G serotype-specific monoclonal antibodies (MAbs) and P and G genotyping by reverse transcription-PCR (RT-PCR) were carried out as described previously (12, 15, 28). Serotyping MAbs were obtained from Serotec Laboratory (Tokyo, Japan) and from Dennis R. Lang (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda,

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TABLE 1. Distribution of rotavirus G serotypes isolated from children with diarrhea in China, by region, 1998–2000

Region	Yr(s) isolated	No. (%) of:							
		Total rotavirus strains isolated	Serotype[a]					Nontypeable strains	Mixed infections
			G1	G2	G3	G4	G9		
Changchun	1998–1999	127	118	4	6	3	0	2	6
	1999–2000	186	174	14	3	1	0	2	8
Qinhuangdao	1998–1999	71	36	3	32	2	0	0	2
	1999–2000	168	126	31	6	7	1	1	4
Beijing	1998–1999	47	35	3	9	1	0	0	1
	1999–2000	70	26	37	8	0	0	0	1
Zhengzhou	1999	81	9	1	70	2	0	0	1
Hangzhou	1998	24	22	1	1	1	0	0	1
	1999	24	19	1	5	0	0	0	1
Fuzhou	1998–1999	81	71	1	2	7	0	1	1
	1999–2000	71	50	12	8	2	0	1	2
Guangzhou	1998	57	53	4	0	0	0	0	0
	1999–2000	75	58	10	9	0	0	0	2
Lanzhou	1999–2000	34	15	5	18	0	2	0	6
Chengdu	1998–1999	6	6	0	0	0	0	0	0
Kunming	1998–1999	39	35	2	4	1	0	0	3
	1999–2000	144	96	29	4	5	9	2	1
Subtotal	1998–1999	452	376 (83.2)	18 (4.0)	54 (11.9)	15 (3.3)	0 (0)	3 (0.7)	14 (3.1)
	1999–2000	853	573 (67.2)	140 (16.4)	131 (15.4)	17 (2.0)	12 (1.4)	6 (0.7)	26 (3.1)
Total	1998–2000	1,305	949 (72.7)	158 (12.1)	185 (14.2)	32 (2.5)	12 (0.9)	9 (0.7)	40 (3.1)

[a] Mixed infections are included in totals.

Md.). Mixed-type specimens from RT-PCR were confirmed with type-specific probes (18). Specimens that could not definitely be assigned a type were analyzed by sequencing of the first-round RT-PCR amplicons with an automated sequencer (ABI 377; Applied Biosystems) and an ABI Prism Big Dye terminator cycle sequencing ready-reaction kit (PE Applied Biosystems). Nucleotide sequences of VP7 and VP8 gene fragments were compared to other sequences in the GenBank database using Seqlab software (Wisconsin Package, version 10.0; Genetics Computer Group, Madison, Wis.), and genotypes were determined by homology to known sequences (data not shown) (5).

Among 3,177 stool specimens screened for rotavirus, 41% of the specimens were positive overall, while 34% (20 to 48%) of the specimens collected in 1998-1999 and 47% (17 to 71%) of those from 1999-2000 were positive (data not shown). Rotavirus was detected primarily in children less than 2 years of age (92%), and only 12% were identified in infants 0 to 5 months of age. Males were nearly twice as likely as females to be screened for rotavirus (ratio, 1.97; range, 1.45 to 3.80).

All 1,305 rotavirus-positive specimens (452 in 1998-1999 and 853 in 1999-2000) were examined for G serotype. Of these, 918 (70%) could be G typed by EIA using type-specific MAbs, leaving 387 specimens (30%) that were G typed by RT-PCR or nucleotide sequencing. Overall, G1 was the most prevalent serotype (72.7%), followed by G3 (14.2%), G2 (12.1%), G4 (2.5%), and G9 (0.9%) (Table 1). These distributions differed by year and region. In total, 9 strains (0.7%) remained nontypeable, and 40 specimens (3.1%) had mixed infections with two or more serotypes.

A total of 327 fecal specimens (123 in 1998 and 204 in 1999) were examined for P genotype (Table 2). Genotypes P[8] (41.6%) and P[4] (35.5%) were predominant, followed by P[6] (9.5%) and P[9] (2.4%). In total, 37 strains (11.3%) remained nontypeable. Only one strain (0.3%) was determined to be a mixed infection (P[8+4]), which was not surprising, since strains with single G types were selected for P genotyping. The four common G-P type combinations (P[8]G1, P[4]G2, P[8]G3, and P[8]G4) accounted for the majority (75.6%) of the all typeable strains. However, when G and P combinations were examined, the diversity of strains was much greater: P[8]G1 was the most common strain (75.7%) but P[4]G1 (10.8%), P[6]G1 (6.3%), and P[9]G1 (7.2%) were also present. Similarly, genotype P[4]G2 was the most common G2 strain, followed by P[8]G2 (5.3%). P[8]G3 was the most common G3 strain (69.7%), followed by P[4]G3 (21.4%) and P[6]G3 (8.9%), and P[8]G4 was the most common G4 strain (66.7%)

TABLE 2. P genotypes of rotavirus among 327 isolates from children with diarrhea in China, by region, 1998–2000

Region	No. (%) of strains					
	With genotype[a]				Non-typeable	With mixed infections
	P[8]	P[4]	P[6]	P[9]		
Changchun	31	7	1	1	7	1
Qinhuangdao	17	23	10	1	3	0
Beijing	12	30	0	0	7	0
Zhengzhou	16	1	0	0	0	0
Hangzhou	7	0	1	2	2	0
Fuzhou	24	13	5	0	6	0
Guangzhou	9	16	1	2	2	0
Lanzhou	3	8	2	0	2	0
Chengdu	5	0	0	1	0	0
Kunming	12	18	11	1	8	0
Total	136 (41.6)	116 (35.5)	31 (9.5)	8 (2.4)	37 (11.3)	1 (0.3)

[a] No P[10] strains were detected.

in 1998 but was displaced by P[6]G4 (87.5%) in 1999; another strain, P[4]G4 (11.8%), was also present. Of 12 G9 strains, 11 were P[6]G9 and the other was P[8]G9.

This work is the first nationwide study in China to document the distribution of rotavirus G and P types and the diversity of the P-G type combinations among children with acute diarrhea. Of note, rotavirus was detected in 41% of all 3,177 children screened for diarrhea at 10 hospitals, underscoring its importance as the most common cause of severe diarrhea in China. Results of this study and prior knowledge of rotavirus strain diversity can provide information important for considering the introduction of vaccines, determining the background of strains in circulation, and predicting changes that might occur when a licensed vaccine is introduced. The first rotavirus vaccine was licensed and introduced in China in 2000, and other vaccines are under development.

Studies characterizing rotavirus strains have been conducted previously in China with strains collected from 1982 to 1997 (7–10, 20, 23, 25, 31–33, 35). These results together with those presented here provide a base of 2,573 strains characterized to date. The serotypes of strains from three regions of China (Changchun, Qinhuangdao, and Beijing) were compared with past results to provide some insight into variability in a single region over time. Although in most years and regions, G1 was the predominant serotype, considerable G-type fluctuations were found from year to year. For example, G2 was the predominant serotype in Changchun in 1986 and in Beijing in 1996 and 1999. G3 was the predominant serotype in Zhengzhou and Lanzhou in 1999, and in Lulong, a county of Qinhuangdao, G3 represented 61.7% of all rotavirus specimens isolated in 1998. Serotype G4 was always a minor strain (3.3% in 1998 and 2.0% in 1999), and 12 G9 strains were isolated in 1999 from three regions (9 from Kunming, 2 from Lanzhou, and 1 from Lulong, Qinhuangdao.)

Overall, the strain distribution in China resembles that in many other developed countries where G1 is the predominant serotype (66.7%), followed by G2 (19.9%), G3 (12.0%), G4 (2.1%), G9 (0.5%), and G8 (0.1%). This pattern varies somewhat from that seen recently in the United States, where G9 is the most common serotype after G1 and G2, and is quite different from that in India, where G9 strains are extremely common and mixed infections are abundant. Of note, G9 strains were identified in three regions of China in 1999, while only one such strain was detected between 1982 and 1997 from Beijing. This increase coincided with an increased global incidence of this strain since 1996 in the various countries. In addition, the recent detection of Chinese G9 strains corresponds to similar results in neighboring countries, such as Thailand and Japan, where G9 strains became more common in 1998.

P typing of 327 strains showed high diversity of G and P combinations. Four P genotypes (P[8], P[4], P[6], and P[9]) were identified, with P[8] and P[4] being the most common and accounting for 86.6% of strains, a distribution similar to that seen worldwide (83.5%) (13). Although genotype P[6] strains were originally isolated from neonates without any symptoms of diarrhea and thought to be avirulent, in recent years they have been shown to be a common cause of gastroenteritis globally (1, 3, 4, 26, 27, 29, 30). In November 1998, a virulent P[6]G4 strain caused an outbreak of neonatal diarrhea in an obstetric ward at a hospital in Beijing (34). In our study, P[6] strains were identified in combination with G9 (35.5%), G4 (25.8%), G1 (22.6%), and G3 (16.1%) serotypes and accounted for 10.7% of all P-typed specimens. In the present study, eight P[9] strains were isolated from children in six regions, and all were serotype G1, in contrast to a single P[9]G3 isolate from Wuhan, Hubei Province, detected in 1995 (25). In total, only 37 (11.3%) specimens were nontypeable by use of genotype-specific primers to five human rotavirus genotypes, suggesting that other P genotypes might be present or that variants of known genotypes could not be amplified with these primers. Overall, 14 different P-G combinations were identified, and none of these reflected the P[12]G[10] characteristics of the Lanzhou lamb rotavirus vaccine. The four common worldwide strains accounted for 75.6% of the total, but the presence of uncommon strains, including the novel types P[4]G3, P[4]G4, and P[8]G2, highlighted the extraordinary diversity of rotaviruses circulating in China.

Our results indicate that vaccines targeting rotavirus diarrhea in China need to protect primarily against the common serotypes, and, if effective, they could decrease the number of hospitalizations of children due to rotavirus gastroenteritis. At the same time, surveillance of strains will be needed to determine if, after vaccination, new variant strains which are not controlled by the vaccine or reassortants that contain gene segments from both the vaccine and natural human strains arise. Typing studies will need to continue to monitor changes in rotavirus strains as vaccines put new pressures on virus selection and evolution.

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