Molecular epidemic features and variation of rotavirus among children with diarrhea in Lanzhou, China, 2001-2006

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Background: **Human rotavirus (HRV) is the most common pathogen causing severe diarrhea among infants and young children worldwide. This study aims to understand rotavirus epidemiology and its variation in the period of 2001-2006 in Lanzhou, Gansu Province, China, and to provide an epidemiological basis for the development of rotavirus vaccine.**

Methods: **A total of 1019 stool specimens were collected from patients with acute diarrhea admitted to the First Hospital of Lanzhou University from 2001 to 2006, who were younger than 5 years old. Dako IDEIATM kits were used for detection of rotavirus, and RT-PCR was performed for determination of G serotype and P genotype of the rotavirus.**

Results: **Rotavirus was present in 51.6% (526) of the 1019 specimens. G serotype identified G3 at 40.9%, G2 14.6%, G1 22.2% and G9 1.9%. Mixed-G infection was observed in 4.4% and non-typeable infection 16.0%. P genotype was observed in 372 samples, of which P[8] accounted for 186 cases (50.0%), P[4] 72 cases (19.4%), mixed-P infection 2 cases (0.5%), and non-typeable cases 112 (30.1%). G3 was the most prevalent G serotype found in this study from 2001 to 2004, G2 was the most prevalent G serotype (34.4%) from 2004 to 2005, and G1 (61.5%) was the most prevalent strain from 2005 to 2006. G9 was detected in 10 cases (1.9%) and G4 was not detected during this 5-year period. P[8] was the most prevalent P genotype found over the 5 consecutive years of this study, although there was a significant transition of P genotype from 2004 to 2005 with P[4] (45%) identified as the predominant P genotype, followed by P[8] (22.1%). The predominant G-P combination was P[8]G1 (33.6%), followed by P[8]G3 (32.1%) and P[4]G2 (17.2%). Rotavirus diarrhea admissions peaked between October and December. Continuous surveillance showed that the incidence rate of rotavirus was the highest in infants aged 6-23 months, averaging 11.0-11.9 months.**

Conclusions: **Five years of continuous surveillance showed that rotavirus remains the most significant viral agent causing diarrhea hospitalization among children under 5 years old in Lanzhou, China although the predominant strain of rotavirus varies between years. Mixed-G serotype infection also appears to occur at a relatively high rate in Lanzhou.**

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***Key words*: diarrhea;**
epidemiology;
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variation

Introduction

Human rotaviruses (HRV) are the most common pathogen causing severe diarrhea among infants and young children worldwide. A recent review estimates that rotavirus infection occurs at least once in children under the age of 5 years.[1] Studies, in China and elsewhere, have shown a shift of predominant rotavirus strain between years and regions and atypical and rare strains have been reported in both developing and developed countries.[2,3] Moreover, a high rate of mixed infections has been detected. All of these factors cause difficulties for further research and the use of vaccines. Hospital-based HRV surveillance systems are able to provide clinical data, and characterize HRV strains, epidemic features and variation. To date, 15 different G serotypes of HRV have been identified,[4,5] including 10 G serotypes that are infective to humans. Globally, G1-G4 are the most prevalent G serotypes, with the incidence and distribution of these 4 G serotypes having seasonal and regional variation. Rotavirus G1 was the most common serotype, followed by G3, G4 and G2 in most Chinese cities in the 1990s.[6] Our study shows the rotavirus molecular epidemiology and its variation in Lanzhou, Gansu Province of China from December

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2001 to June 2006, providing scientific support for the development of a higher quality rotavirus vaccine.

Methods

We enrolled and collected 1019 stool specimens from in-patients who had acute diarrhea under 5 years old at the Department of Pediatrics of the First Hospital of Lanzhou University from December 2001 to June 2006.

Stool samples were tested for the detection of rotavirus antigen using commercial immunoassay kits (IDEIA™ Rotavirus kit, DAKO Cytomation., Ely, UK). Rotavirus positive specimens were further categorized into strain as G serotype and P genotype by reverse-transcriptase polymerase chain reaction (RT-PCR), as described by Fang et al.[7] Rotavirus dsRNA was extracted from stool with Trizol reagent according to the protocol provided by the manufacturer. The dsRNA samples were then subjected to 1 cycle of reverse transcription (RT) (42°C, 60 minutes) followed by 30 cycles of PCR. Each PCR cycle for G serotype or P genotype contained steps of 1 minute at 94°C, 1 minute at 42°C, and 1 minute at 72°C, then elongated 7 minutes at 72°C. The PCR products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide. Primer sequences are described in Table 1.

Statistical analysis

The Chi-square test or Fisher's exact test was used for statistical analysis when appropriate using SPSS 11.0. $P<0.05$ was considered statistically significant.

Results

Rotavirus detection and characterization of G serotype and P genotype

Of the 1019 stool samples collected from inpatients with acute diarrhea under 5 years old at the Department of Pediatrics, the First Hospital of Lanzhou University from December 2001 to June 2006, 526 (51.6%) were found rotavirus-antigen positive. Rotavirus-positive specimens were further characterized by G serotype identifying G3 in 40.9% of the cases (215/526), G2 in 14.6% (77/526), G1 in 22.2% (117/526), G9 in 1.9% (10/526), and mixed-G in 4.4% of cases (23/526). Sixteen percent (84/256) of the cases were G non-typeable. A representative subset of the 372 isolates was also P-typed. P[8] was found in 50.0% of the 372 cases (186/372), P[4] in 19.4% (72/372), mixed-P in 0.5% (2/372) and non-typeable in 30.1% (112/372). The Chi-square test showed that the rates of rotavirus antigen detection were significantly different during the periods of 2001-2002 and 2003-2004 ($\chi^2=3.94$, $P<0.05$), 2001-2002 and 2005-2006 ($\chi^2=10.43$, $P<0.01$) and 2004-2005 and 2005-2006 ($\chi^2=11.35$, $P<0.01$)

Yearly distribution of HRV G serotype and P genotype

G3 was the most prevalent G serotype found in this study during the periods of 2001-2002 and 2003-2004, accounting for 58.7% and 68.1% of the cases respectively. G2 accounted for only 4.6% and 2.8%, and the rare G9 strain accounted for 2.8% and 4.2% during the same periods. G1 was the second most prevalent strain during the period of 2001-2002, accounting for 18.3%, but it was not detected during the period of 2003-2004. G2 was identified as the most prevalent serotype for 34.4% from 2004 to 2005, with G3 for 32.8%, G1 for 1.1% (in only 2 cases), and G9 was not detected. G1 was identified as the most prevalent serotype during the period of 2005-2006 in 61.5% of the cases and G9 in 2.6% of the cases. G4 was not detected during the entire 5 years of consecutive surveillance. Mixed-G infections accounted for 6.4%, 5.8% and 3.2% during the periods of 2001-2002, 2004-2005 and 2005-2006 respectively, but were not found during the period of 2003-2004. P[8] was the most

Table 1. Primers used in genotyping

Primer	G/P typing	Location	Sequence (5'-3')	Product size (bp)
4Con3	P (+)	11-32	TGGCTTCGCCATTTTATAGACA	-
4Con2	P (-)	868-887	ATTTCGGACCATTTATAACC	877
1T1	P[8] (-)	339-356	TCTACTTGGATAACGTGC	346
2T1	P[4] (-)	474-494	CTATTGTTAGAGGTTAGAGTC	484
3T1	P[6] (-)	259-278	TGTTGATTAGTTGGATTCAA	268
4T1	P[9] (-)	385-402	TGAGACATGCAATTGGAC	392
5T1	P[10] (-)	575-594	ATCATAGTTAGTAGTCGG	584
9Con1	G (+)	37-56	TAGCTCCTTTTAATGTATGG	-
9Con2	G (-)	922-941	GTATAAAATACTTGCCACCA	905
9T1	G1(-)	176-195	TCTTGTCAAAGCAAATAATG	159
9T2	G2 (-)	262-281	GTTAGAAATGATTCTCCACT	245
9T3	G3 (-)	484-503	GTCCAGTTGCAGTGTTAGC	467
9T4	G4 (-)	423-440	GGGTCGATGGAAAATTCT	404
9T9B	G9 (-)	131-147	TATAAAGTCCATTGCAC	111

prevalent P genotype during the periods of 2001-2002, 2003-2004 and 2005-2006, accounting for 62.5%, 33.3%, and 75.6%, respectively. P[4] (45%) was the predominant P genotype from 2004 to 2005, followed by P[8] (22.1%). No other P genotypes were observed (Table 2). The predominant G-P combination was P[8]G1, P[8]G3 and P[4]G2, accounting for 33.6%, 32.1% and 17.2% of all combination cases, respectively. Other G-P combinations were P[4]G3 (5.0%), P[8]G2 (1.5%), P[8]G9 (1.9%), P[4]G1 (1.1%) (Table 3). The 5-year consecutive surveillance showed that the most prevalent G-P combination was P[8]G3 before 2004, P[4]G2 between 2004 and 2005, and P[8]G1 between 2005 and 2006.

Age and gender distributions of rotavirus patients

Over the 5-year period the detection rates of rotavirus were the highest among infants of 6-23 months old. The mean age of the infants was 11.0-11.9 months, and there was no significant gender difference. The cumulative percentage of rotavirus incidence was 64.6% for those who were younger than 1 year and 95.6% for those who were younger than 2 years. No statistical correlation was observed between the detection rates in the same age group in different years (Fig.).

Seasonal distribution of rotavirus infections

During the study period of 2001-2006, cases of rotavirus induced diarrhea peaked between October and December in Lanzhou (Table 4) at a statistical significant level (χ^2=39.72, P<0.001). Within this period the peaked season of rotavirus diarrhea incidence varied between years. During the periods of 2001-2002 and 2003-2004, the incidence peaked between October and November, with a detection rate of rotavirus for 61.5% and 79.5% respectively. From 2004 to 2005, the incidence of rotavirus diarrhea peaked between October and December; the detection rate was 56.8% during the period of 2005-2006, and the incidence peaked between September and December.

Table 2. G serotype and P genotype of rotavirus during the period of 2001-2006

Types	Periods of years (%)			
	2001-2002	2003-2004	2004-2005	2005-2006
G1	20 (18.3)	0	2 (1.1)	96 (61.5)
G2	5 (4.6)	2 (2.8)	65 (34.4)	5 (3.2)
G3	64 (58.7)	49 (68.1)	62 (32.8)	39 (25.0)
G9	3 (2.8)	3 (4.2)	0	4 (2.6)
G-NT	10 (9.2)	18 (24.9)	49 (25.9)	7 (4.5)
G-MX	7 (6.4)	0	11 (5.8)	5 (3.2)
P[8]	25 (62.5)	12 (33.3)	31 (22.1)	118 (75.6)
P[4]	3 (7.5)	0	63 (45)	6 (3.8)
P-NT	11 (27.5)	24 (66.7)	46 (32.9)	31 (19.9)
P-MX	1 (2.5)	0	0	1 (0.64)

G-NT: G non-typeable; G-MX: mixed-G; P-NT: non-typeable; P-MX: mixed-P.

Table 3. G-P combinations of rotavirus during the period of 2001-2006

Types	P[4] (%)	P[8] (%)	P[8] + P[4] (%)	Total
G1	3 (1.1)	88 (33.6)	1 (0.4)	92
G2	45 (17.2)	4 (1.5)	1 (0.4)	50
G3	13 (5.0)	84 (32.1)	0	97
G9	0	5 (1.9)	0	5
G2+G3	10 (3.8)	3 (1.1)	0	13
G3+G1	1 (0.4)	4 (1.5)	0	5
Total	72	188	2	262

Discussion

Consecutive surveillance from 2001 to 2006 showed that the incidence of rotavirus diarrhea was 51.6% in children under 5 years of age in Lanzhou, China, which is similar to that reported elsewhere.[8,9]

Serotype and genotype distribution of rotavirus infections in Lanzhou

Serotype G3 was predominant in rotavirus strains in

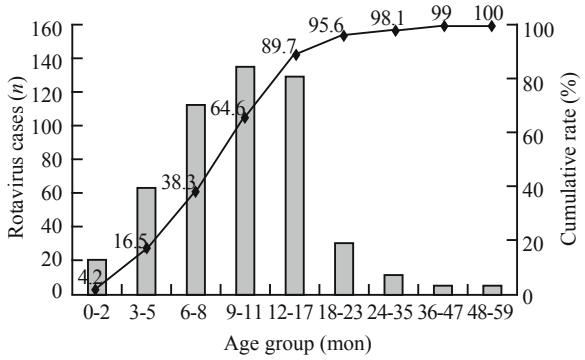

Fig. Age distribution of rotavirus diarrhea during the period from December 2001 to June 2006

Table 4. Seasonal distribution of rotavirus diarrhea during the period of 2001-2006

Month	Number	Rotavirus positive *n* (%)
January	70	32 (45.7)
February	32	13 (40.6)
March	46	17 (37.0)
April	51	23 (45.0)
May	45	17 (37.8)
June	41	14 (34.1)
July	46	15 (32.6)
August	105	39 (37.1)
September	101	57 (56.4)
October	215	142 (66.0)
November	166	106 (63.9)
December	101	51 (50.5)
Total	1019	526 (51.6)

6 hospitals of China from 2001 to 2003.[10] Although serotype G3 accounted for about 60% of all rotavirus strains from 2001 to 2004 in Lanzhou, serotype G3 was not the predominant G serotype for the years after 2004.[11] During the period from 2004 to 2005, serotype G2 was the most prevalent (34.4%), followed by G3 serotype for 32.8%. From 2005 to 2006, it was replaced by serotype G1, accounting for 61.5%, whereas serotype G3, 25.0%. Serotype G4 is considered common in all rotavirus strains worldwide. However, it has been detected at a rate as low as 3.5% in 8 cities in China from 1998 to 1999.[6] Serotype G4 was not detected during this study period in Lanzhou. Serotype G9 has been detected around the world since the first case was confirmed in 1983[12] and reported in China in 1994.[13] More recently, serotype G9 has been detected in different regions of China at rates between 1% and 5.6%.[6,14,15] Zhang et al[16] reported serotype G9 infection in nine cities of China from 1998 to 2004. Among 1268 rotavirus-positive specimens, serotype G9 accounted for 3.5%. Most of the cases (34/45) occurred in Kunming city, followed by Lanzhou (8/45), Changchun (2/45), and then Lulong (1/45). However, no G9 was identified in Beijing, Zhengzhou, Hangzhou, Fujian, and Guangzhou. This study showed that serotype G9 was present at a rate of 1.9% from 2001 to 2006 in Lanzhou, which is similar to the earlier reports.[6,15,16] Over this period, the duration with the highest rate of serotype G9 varied from 2003 to 2004, no serotype G9 was identified from 2004 to 2005 in Lanzhou. Further research and confirmation are needed for the serotype G9 strain in this area.

G-mixed infection is common in some developing countries,[17,18] but it is lower in China, at approximately 0.8%-3.9%. A 5-year study revealed about 4.4% mixed infection, which was higher than other regions in China, but lower than India (21%)[18] and Bengal (23%).[19] Mixed infection provides a chance for serotype recombination, thereby enabling serotype changes. Because the percentage of mixed infection is higher in Lanzhou than other regions in China, further epidemic surveillance is required.

The non-typeable rotavirus strains may be due to the accumulation of VP7 gene segment mutations.[20] Rare strains are frequently detected in other countries,[21,22] and those non-typeable samples in this study could represent other G serotypes such as G5, G8, G10 and G12, which we were difficult to characterize. Of particular relevance, serotype G5 and G8 viruses appear to be epidemiologically significant, becoming the predominant strain in children with diarrhea in some areas.[23] Serotype G5 was originally identified in humans in Asia.[24]

P[8] and P[4] are the most prevalent P genotype in the world.[25-27] But P[8] was the most prevalent genotype in HRV patients in Lanzhou throughout 2001 to 2006, except for the year from 2004 to 2005. P[4] was the second most prevalent genotype because of its frequent combination with serotype G2. Among the combinations of G serotype and P genotype, P[8]G1, P[8]G3, P[4]G2, and P[8]G4 are common G-P type combinations worldwide. P[8]G1, P[8]G3 and P[4]G2 were predominating which accounted for 82.2% of all combinations from 2001 to 2006 in this study.

Season and age distribution in relation to rotavirus infections in Lanzhou

Although rotavirus is detected year around, there are obvious seasonal trend and regional variations in the incidence of rotavirus infection. In general, the peak incidence of rotavirus diarrhea is in autumn and winter of different zone in different countries.[27] These findings have been reported in America, Canada, Spain, Poland, Japan, Vietnam,[28,29] and China. The present study showed that the peak season for rotavirus diarrhea was between October and December, which was similar in Kunming.[8] During the period of 2005-2006, the peak season for rotavirus diarrhea was between September and December, a little bit earlier than that of past years in Lanzhou, but similar to that in Vietnam.[27] The highest rate of rotavirus infection in children of 6 to 23 months of age was similar to that reported elsewhere.[8,28,29] This study showed in the period of 2001-2006, 95.6% of HRV infections cumulatively occurred in infants younger than 2 years. The age distribution related to the peak incidence of rotavirus infection coincides with the decline of maternal antibodies and the immature immune system in this age range, which make the infants susceptible to rotavirus infection. Hence it is necessary to further molecular epidemiological study on HRV and to develop new strategies of prevention according to the changes of predominant rotavirus strains. For the development and evaluation of a vaccine, prompt survey should be done to find out variations of prevalent serotypes and the emergence of rare types or new strains. Our study illustrates the diversity and complexity of rotavirus strains in cyclic of occurrence in Lanzhou.[30-32] The consecutive surveillance for 5 years may be helpful to the development of rotavirus vaccines and vaccine-mediated immune protection of susceptible children in Lanzhou.

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References

1 Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9:565-572.
2 Shinozaki K, Okada M, Nagashima S, Kaiho I, Taniguchi K. Characterization of human rotavirus strains with G12 and P[9] detected in Japan. J Med Virol 2004;73:612-616.
3 Martella V, Ciarlet M, Bányai K, Lorusso E, Cavalli A, Corrente M, et al. Identification of a novel VP4 genotype carried by a serotype G5 porcine rotavirus strain. Virology 2006;346:301-311.
4 Rivest P, Proulx M, Lonergan G, Lebel MH, Bédard L. Hospitalisations for gastroenteritis: the role of rotavirus. Vaccine 2004;22:2013-2017.
5 Iturriza-Gómara M, Green J, Brown DW, Ramsay M, Desselberger U, Gray JJ. Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. J Clin Microbiol 2000;38:4394-4401.
6 Fang ZY, Qi J, Yang H, Wang CX, Ye Q, Ma L, et al. Serotype and genotype study of childhood rotaviruses isolated in 1998-1999 in China. Chin J Virol 2001;17:17-23.
7 Fang ZY, Jin SJ, Qin SM, Zhao XF, Wu D, Shijima HU, et al. Serotypes of group A rotavirus isolates determined by PCR in Hebei and Henan provinces, China. Chin J Virol 1994; 10:316-321.
8 Zhang LJ, Du ZQ, Zhang Q, Kang HY, Zheng LSH, liu XM, et al. Rotavirus surverillance date from Kunming Children's Hospital, 1998-2001. Chin J Epidemiol 2004;25:396-399.
9 Gil A, Carrasco P, Jiménez R, San-Martín M, Oyagüez I, González A. Burden of hospitalizations attributable to rotavirus infection in children in Spain, period 1999-2000. Vaccine 2004;22:2221-2225.
10 Fang ZY, Wang B, Kilgore PE, Bresee JS, Zhang LJ, Sun LW, et al. Sentinel hospital surveillance for rotavirus diarrhea in the People's Republic of China, August 2001-July 2003. J Infect Dis 2005;192 Suppl 1:S94-S99.
11 Ye XH, Jin Y, Fang ZY, Song YP, Xie HP, Zhang Q, et al. Etiological study on viral diarrhea among children in Lanzhou, China, 2004-2005. Chin J Epidemiol 2006;27:117-122.
12 Clark HF, Hoshino Y, Bell LM, Groff J, Hess G, Bachman P, et al. Rotavirus isolate WI61 representing a presumptive new human serotype. J Clin Microbiol 1987;25:1757-1762.
13 Qian Y, Yuan LJ, Xiong CH, Zhang Y, Liu J, Guan DH, et al. Identification of rotavirus G9 type from a stool specimen collected from a child with diarrhea in Beijing. Chin J Virol 1994;10:263-267.
14 Tian JM, Xu L, Sheng H, Jin H. Study on the clinical epidemiology of young children under 5 years old diarrhea caused by rotavirus in Suzhou area. Pediatr Emerg Med 2003; 10:370-371.
15 Fang ZY, Yang H, Qi J, Zhang J, Sun LW, Tang JY, et al. Diversity of rotavirus strain among children with acute diarrhea in China: 1998-2000 surveillance study. J Clin Microbiol 2002;40:1875-1878.
16 Zhang Q, Duncan S, Roger G, Fang ZY, Wang DK, Ye XH, et al. Molecular epidemiological research on rotavirus serotype G9 isolated from 9 regions in China. Chin J Vaccines Immunization 2006;12:476-479.
17 Bahl R, Ray P, Subodh S, Shambharkar P, Saxena M, Parashar U, et al. Incidence of severe rotavirus diarrhea in New Delhi, India, and G and P types of the infecting rotavirus strains. J Infect Dis 2005;192 Suppl 1:S114-119.
18 Jain V, Das BK, Bhan MK, Glass RI, Gentsch JR. Indian Strain Surveillance Collaborating Laboratories. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infections in India. J Clin Microbiol 2001;39:3524-3529.
19 Unicomb LE, Podder G, Gentsch JR, Woods PA, Hasan KZ, Faruque AS, et al. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. J Clin Microbiol 1999;37:1885-1891.
20 Moon SS, Green YS, Song JW, Ahn CN, Kim H, Park KS, et al. Genetic distribution of group A human rotavirus types isolated in Gyunggi province of Korea, 1999-2002. J Clin Virol 2007;38:57-63.
21 Kheyami AM, Nakagomi T, Nakagomi O, Dove W, Hart CA, Cunliffe NA. Molecular epidemiology of rotavirus diarrhea among children in Saudi Arabia: first detection of G9 and G12 strains. J Clin Microbiol 2008;46:1185-1191.
22 Le VP, Kim JY, Cho SL, Nam SW, Lim I, Lee HJ, et al. Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. J Med Virol 2008;80:175-182.
23 Matthijnssens J, Rahman M, Yang X, Delbeke T, Arijs I, Kabue JP, et al. G8 rotavirus strains isolated in the Democratic Republic of Congo Belong to the DS-1-Like Genogroup. J Clin Microbiol 2006;44:1801-1809.
24 Duan ZJ, Li DD, Zhang Q, Liu N, Zheng LS, Tang JY, et al. Discovery of a human group A rotavirus strain with G5 specificity in Lulong County of China. Chin J Virol 2007;23:96-101.
25 Macedo CI, Christofoletti A, Munford V, Rácz ML. G and P rotavirus genotypes in stool samples from children in Teresina, State of Piauí. Rev Soc Bras Med Trop 2007;40:381-384.
26 Fang ZY, Zhang LJ, Tang JY, Zhang Q, Hu HK, Xie HP, et al. Rotavirus diarrhea among children in Lulong County, Hebei Province, China. Chin J Virol 2005;21:21-25.
27 Castello AA, Arvay ML, Glass RI, Gentsch J. Rotavirus strain surveillance in Latin America: a review of the last nine years. Pediatr Infect Dis J 2004;23:S168-172.
28 Nguyen TV, Le Van P, Le Huy C, Weintraub A. Diarrhea caused by rotavirus in children less than 5 years of age in Hanoi, Vietnam. J Clin Microbiol 2004;42:5745-5750.
29 Suzuki H, Sakai T, Tanabe N, Okabe N. Peak rotavirus activity shifted from winter to early spring in Japan. Pediatr Infect Dis J 2005;24:257-260.
30 Zhang CF, Jin Y, Zhang Y, Qian Y. VP4 and VP7 typing for rotaviruses identified from stool specimens collected from infants and young children with acute diarrhea in Lanzhou. Chin J Pediatr 2002;40:409-412.
31 Dong QL, Jin Y, Zang Q, Xie HP, Fang ZY. Clinical study on the characteristic patterns of the diarrhea caused by rotavirus and calicivirus in infants and young children in Lanzhou. J Clin Pediatr 2005;23:364-367.
32 Jin Y, Huang X, Fang ZY, Tan JY, Xie HP, Wang DK, et al. Molecular epidemiology of virus diarrhea among infants and young children in Lanzhou. Chin J Pract Pediatr 2006;21:15-18.

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