

Laboratory and Epidemiology Communications

Serotyping of Human Group A Rotaviruses in Nara Prefecture, Japan

Yoshiteru Kitahori*, Yumiko Inoue, Hisakatsu Takebe and Shunsuke Imai

Department of Virology, Nara Prefectural Institute for Hygiene and Environment, Nara 630-8131

Communicated by Takashi Kawamura

(Accepted April 9, 2003)

Rotaviruses, together with eight other distinct genera, are members of the family *Reoviridae*. Eleven segments of double-strand RNA are packaged within a core shell surrounded by a double-layered capsid. The outer capsid contains the major neutralization antigen VP7. The VP7 protein defines the serotype G. In group A rotaviruses, 14 distinct G serotypes have been recognized by neutralization assay. At least 10 G serotypes have been found in humans. The

epidemiological importance of the four major G types (G-1, 2, 3, and 4) has been indicated by their worldwide distribution. The importance of the newly described G serotypes 8, 9, and 12 remains unknown. In the present study, we have carried out G-serotyping, using ELISA and RT-PCR methods as type-specific assays, of human rotaviruses in the winter seasons in Nara Prefecture between 1999 and 2002.

Fecal specimens were collected from children aged from 3-months to 13-years-old complaining of acute gastroenteritis. A total of 117 rotavirus-positive fecal specimens, which had been screened using a latex agglutination kit (ROTA-ADENO DRY, Orion Diagnostica, Espoo, Finland) were obtained from Saiseikai-Gose Hospital, Saiseikai-Nara Hospital, Mimuro

*Corresponding author: Mailing address: Department of Virology, Nara Prefectural Institute for Hygiene and Environment, Ohmori-cho 57-6, Nara 630-8131, Japan. Tel: +81-742-20-2887, Fax: +81-742-27-0634, E-mail: y.kitahori@ihe.pref.nara.jp

Table 1. Serotyping of group A rotavirus in Nara Prefecture between 1999 and 2002

	1999	2000	2001	2002	Total
No. of specimens	16	37	23	41	117
No. of specimens with serotype	11	34	18	36	99
Serotype 1	10 (91)	27 (79)	12 (67)	19 (53)	68 (69)
2	0	7 (21)	6 (33)	6 (17)	19 (19)
3	0	0	0	4 (11)	4 (4)
4	0	0	0	7 (19)	7 (7)
9	1 (9)	0	0	0	1 (1)
Not type	5	3	5	5	18

Numbers in parentheses indicate percentages.

Hospital, Kokuho-Central Hospital, Yaoi Children's Hospital, Yamaga Children's Hospital, Yamamoto Children's Hospital, and Okamoto Children's Hospital. The fecal specimens were suspended from 5 to 10% in Hank's solution and were centrifuged. The supernatants were used for serotyping by ELISA and RT-PCR. ELISA assay was done using a ROTA-MA kit (Serotec, Sapporo), which used G1-4 monoclonal antibodies against the human rotavirus. RT-PCR assay was carried out according to the protocol of Taniguchi et al. (1). The results of serotyping the rotaviruses in each season are summarized in Table 1. ELISA assay was able to identify 45 (39%) of 117 specimens, i.e., 36 of serotype G1, 4 of serotype G2, 2 of serotype G3, and 3 of serotype G4. PCR assay identified 87 (74%) of 117 specimens, i.e., 58 of serotype G1, 18 of serotype G2, 4 of serotype G3, 6 of serotype G4, and 1 of serotype G9. Thus, RT-PCR assay could serotype more isolates than could ELISA assay. Finally, 99 specimens were classified into five different serotypes; G1 (68, 69%), G2 (19, 19%), G4 (7, 7%), G3 (4, 4%), and G9 (1, 1%). Eighteen other specimens, however, could not be typed.

In the winter seasons of 2000 and 2001, serotypes G1 and G2 were detected, and the incidence of respective serotype was 27 (79%) and 7 (21%) in 2000, and 12 (67%) and 6 (33%) in 2001. In 1999, serotype G1 and G9, at 10 (91%) and 1 (9%), respectively, were detected. In 2002, interestingly, four serotypes, serotype G1 (19 isolates, 53%), serotype G2 (6 isolates, 17%), serotype G3 (4 isolates, 11%), and serotype G4 (7, 19%), were found. Thus, G1 and G2 were the main serotypes in Nara Prefecture from 1999 to 2002. Figure 1

shows the age distribution of the serotypes. The incidence of infection was high at 1-2 years of age. In the 90 children (<1-5 years old), the frequency of serotypes G1 and G2 was 66 (73%) and 13 (14%), respectively. But the incidence of G1-serotype in school-aged children (6-13 years old) was not significantly high: there were 2 G1 (22%), 6 G2 (67%), and 1 G4 (11%).

Severe diarrhea is the frequent symptom in children. More than 90% of children are infected with rotavirus by 3 years of age. The distribution pattern of serotypes was almost the same as in the previous reports except for serotype G9. Only one G9 strain was identified in the 1999 season (a 1-year-old boy in the central area of Nara Prefecture). However, the prevalence of the G9 strain in Japan has been reported by Zhou et al. (2); in 1998-1999, G9 was the most prevailing serotype with frequencies of 53% in Tokyo and 71% in Sapporo. The G9 strain was fourth in the isolation frequency ranking among G1, G2, G9, and G3 isolated in the United States in 1996-1997 (3). Recently, however, the incidence of serotype G9 has increased in children from India, Bangladesh, France, Australia, and the United Kingdom. In our data, the predominant serotype among isolates from children at 6-13 years (school-aged children) was serotype G2 (67%) (Table 2). Griffin et al. (4) reported that in their study rotavirus isolated from adults were serotype G2 in all the outbreaks. Serotype G2 was found in outbreaks of gastroenteritis among school-aged children (7-8 age) and adults at 30-80 years old in Japan during 2000 (5,6). These reports strongly suggest that rotavirus infection in adults and school-aged children often

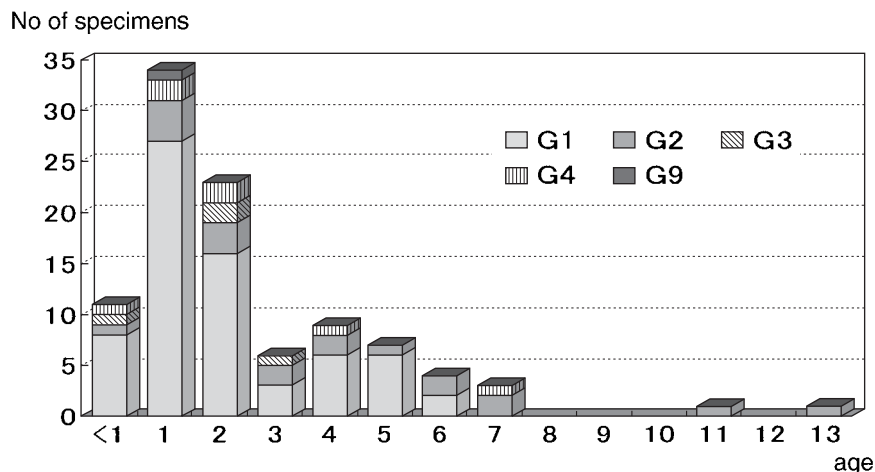


Fig. 1. Age distribution of rotavirus serotypes, G1-G9, from 1999 to 2002 in Nara Prefecture.

Table 2. Comparison of age distribution and G-serotype

Age	0-5	6-13
No. of specimens with serotype	90	9
Serotype 1	66 (73)	2 (22)
2	13 (14)	6 (67)
3	4 (4)	0
4	6 (7)	1 (11)
9	1 (1)	0

Numbers in parentheses indicate percentage.

involve the serotype G2 rather than the G1.

REFERENCES

1. Taniguchi, K., Wakasugi, F., Pongsuwanna, Y., Urasawa, T., Ukae, S., Chiba, S. and Urasawa, S. (1992): Identification of human and bovine rotavirus serotypes by polymerase chain reaction. *Epidemiol. Infect.*, 109, 303-312.
2. Zhou, Y., Li, L., Kim, B., Kaneshi, K., Nishimura, S., Kuroiwa, T., Nishimura, T., Sugita, K., Ueda, Y., Nakaya, S. and Ushijima, H. (2000): Rotavirus infection in children in Japan. *Pediatr. Int.*, 42, 428-439.
3. Griffin, D. D., Kirkwood, C. D., Parashar, U. D., Woods, P. A., Bresee, J. S., Glass, R. I., Gentsch, J. R. and the National Rotavirus Strain Surveillance System Collaborating Laboratories (2000): Surveillance of rotavirus strains in the United States: identification of unusual strains. *J. Clin. Microbiol.*, 38, 2784-2787.
4. Griffin, D. D., Fletcher, M., Levy, M. E., Ching-Lee, M., Nogami, R., Edwards, L., Peters, H., Montague, L., Gentsch, J. R. and Glass, R.I. (2002) Outbreaks of adult gastroenteritis traced to a single genotype of rotavirus. *J. Infect. Dis.*, 185, 1502-1505.
5. Asakawa, H., Machida, A., Ozawa, S., Yokoyama, H. and Yamagami, T. (2000): An outbreak of group A rotavirus gastroenteritis at a home for the aged, March-April 2000-Yamanashi. *Infect. Agents Surveillance Rep.*, 21, 144 (in Japanese).
6. Shinozaki, K., Okada, M., Kaiho, I. and Fukuhara, M. (2000): An outbreak of diarrheal disease due to group A rotavirus at a primary school, March 2000-Chiba. *Infect. Agents Surveillance Rep.*, 21, 145 (in Japanese).