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Subgroup and Serotype Distributions of Human, Bovine, and Porcine Rotavirus in Thailand

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The subgroup and serotype specificities of human, bovine, and porcine group A rotaviruses in stool specimens collected in Thailand were examined by an enzyme-linked immunosorbent assay by using subgroup- and serotype-specific monoclonal antibodies. A clear yearly change was observed in the serotype distribution of human rotavirus. Between 1983 and 1984, serotype 4 was the most prevalent, while the highest frequency of serotype 2 was found between 1987 and 1988. All the bovine and porcine rotaviruses examined showed subgroup I specificities and long RNA patterns. It was of note that serotype 3 porcine rotaviruses were found at a high frequency.

Rotavirus has been isolated in various species of young animals and human infants (8, 15). The rotavirus has two distinct antigenic specificities: subgroup and serotype. Subgroup specificity is associated with a major component of the inner capsid (VP6), which is encoded by RNA segment 6. Two subgroups (I and II) have been described, and the presence of a third subgroup has also been proposed (17, 25, 29). Serotype specificity defined by the neutralization test is associated with an outer capsid protein (VP7) encoded by RNA segment 8 or 9. By cross-neutralization tests, seven distinct rotavirus serotypes have been established among mammalian, including human, and avian strains (14, 30). In addition, several new candidate serotypes have been described recently (6, 19, 24). VP4 (formerly designated VP3), which is encoded by RNA segment 4, also contains an independent neutralization antigen (13, 18), but this protein appears to be mainly responsible for heterotypic cross-reactivity (26).

Diarrheal diseases are responsible for 5 million to 10 million deaths per year in infants and young children in developing countries, where malnutrition is common (15). Among the various pathogens, rotavirus is the major cause of severe diarrhea. Therefore, development of an effective vaccine against rotavirus is urgently needed to control rotavirus diarrhea. However, the presence of multiple serotypes in human rotaviruses (HRVs) have hampered the development of a broadly protective HRV vaccine (4, 16). The extensive vaccine trials with rhesus rotavirus (serotype 3 simian virus) suggest that protection by the vaccine appears to be serotype specific (16), although the recent WC3 (serotype 6 bovine virus) vaccine trials showed an effective protection against serotype 1 rotavirus infection (5). Thus, it seems evident that the survey on serotype distributions in various regions is indispensable for providing fundamental data useful for vaccine development and evaluation.

For determining the subgroup and serotype specificities of rotavirus in stool samples, a rapid and simple enzyme-linked immunosorbent assay (ELISA) system with specific monoclonal antibodies (MAbs) has recently been developed (27, 29), and the validity of the method has been confirmed (31). In this study, the subgroup and serotype distributions of human, bovine, and porcine rotavirus strains in Thailand were surveyed by the use of the ELISA with specific MAbs. We also used RNA pattern analysis and electron microscopy to explore the reason for the failure of subgroup or serotype determination of some samples.

MATERIALS AND METHODS

Fecal specimens. A total of 543 human stool specimens were collected from infants (ages, 8 h after birth to 1 year and 7 months) and adults (ages, 24 to 48 years) with acute gastroenteritis who were admitted to Childrens Hospital, Prapinglao Hospital, and Bamrajnaradul Hospital in Bangkok, Thailand, between 1983 and 1984 and between 1987 and 1988. A total of 43 bovine fecal specimens were obtained from calves with diarrhea in two dairy herds in Saraburi and Nakornrachasima Provinces in Thailand, and 18 porcine fecal specimens were obtained from piglets with diarrhea in a herd in Rachaburi Province in Thailand. An approximately 10% stool suspension was made in Eagle minimal essential medium by rigorous homogenization in a Vortex mixer for 2 min and was clarified by low-speed centrifugation (2,000 × *g* for 20 min).

MAbs. Anti-VP6 YO-156 antibody, which commonly recognizes group A rotaviruses, was used to detect group A rotaviruses. For the subgrouping assay, subgroup I-specific S2-37 and subgroup II-specific YO-5 antibodies directed to VP6 were used. For the serotyping assay, the following four anti-VP7 serotype-specific neutralizing MAbs (N-MAbs) were used: serotype 1-specific KU-6BG, serotype 2-specific S2-2G10, serotype 3-specific YO-1E2, and serotype 4-specific ST-2G7. In addition, an anti-VP4 YO-2C2 antibody, which is commonly reactive with a neutralization epitope on the outer capsid of group A rotaviruses, was included for judging the amount of double-shelled particles. The properties of the MAbs listed above have been described previously (26–29).

ELISA. For determining the subgroup and serotype specificities of the rotavirus, an ELISA with MAbs was used. The ELISA with MAbs was performed as described previ-

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TABLE 1. Subgroup and serotype distributions of HRV in Thailand

Yr of collection	Subgroup	No. of strains with the following serotypes:					
		1	2	3	4	Not determined	Total
1983 to 1984	I	0	4	0	0	1	5
	II	7	0	0	21	11	39
	Not determined	0	1	0	0	4	5
1987 to 1988	I	0	12	0	0	3	15
	II	11	0	0	2	5	18
	Not determined	0	0	0	0	6	6

ously, with some modifications (27, 29; S. Urasawa, T. Urasawa, K. Taniguchi, F. Wakasugi, N. Kobayashi, S. Chiba, N. Sakurada, M. Morita, O. Morita, M. Tokieda, H. Kawamoto, Y. Minekawa, and M. Ohseto, J. Infect. Dis., in press). The wells of polyvinyl microtiter plates were coated with ascitic fluid diluted 1:10,000 in 10 mM phosphate-buffered saline (pH 7.5) for 1 day at 4°C. After they were washed with phosphate-buffered saline containing 0.05% Tween, the wells were incubated with 1% bovine serum albumin in phosphate-buffered saline containing 0.05% Tween for 1 day at 4°C and subsequently washed. A mixture of a 10% stool suspension (37.5 μl) and 10% skim milk (12.5 μl) was allowed to react in the wells overnight at 4°C. After it was washed, 50 μl of rabbit anti-HRV serum (1:5,000 dilution) was added and incubated for 1 h at 37°C. After the washing, 50 μl of peroxidase-conjugated goat anti-rabbit immunoglobulin G (Chemical Credential ICN Immunological) was added. The plates were incubated for 1 h at 37°C and washed. The reaction with the substrate *o*-phenylenediamine was allowed to develop for 30 min at room temperature, and then it was stabilized by the addition of 25 μl of 3 N sulfuric acid. The optical density was measured at 492 nm with a micro-ELISA reader (EAR; SLT-Labinstrument, Salzburg, Austria).

RNA electropherotyping. Viral RNA was extracted from fecal samples with phenol-chloroform (11). Electrophoresis of RNA was conducted at room temperature for 1.5 h at 40 mA with 1.0-mm-thick polyacrylamide mini-gels (8.5 by 9.0 cm). The gels were stained with silver.

Electron microscopy. Virus was pelleted through a 25% sucrose cushion by ultracentrifugation. The pellet was suspended in distilled water, placed on a Formvar-coated grid, and negatively stained with 3% uranyl acetate. The grids were examined with an electron microscope (EM H-600; Hitachi).

RESULTS

Subgroup and serotype distributions of HRVs in Thailand. A total of 88 stool specimens positive for group A HRV were subjected to subgroup and serotype determinations. A total of 77 (87.5%) samples could be subgrouped: 20 belonged to subgroup I and 57 belonged to subgroup II. Rotavirus with subgroup I specificity exhibited a short RNA pattern, while rotavirus with subgroup II specificity had a long RNA pattern. A clear difference in subgroup distribution was found in samples collected in different years. Between 1983 and 1984, the ratio of subgroup I to subgroup II was 0.13; in contrast, between 1987 and 1988 the ratio was 0.83 (Table 1). The subgroup specificities of 10 samples could not be determined. Of these, five samples reacted with both subgroup I- and II-specific MAbs. However, two specimens seemed to contain two different rotavirus strains on the basis of the result of RNA electropherotyping (a mixture of short and long RNA patterns). One sample appeared to show a nonspecific reaction, since the sample reacted to bovine serum albumin in the ELISA system. The reason for the reactivity of the remaining two samples (samples 122 and 124) with both subgroup I- and II-specific MAbs was unknown, since they did not contain enough RNA for RNA pattern analysis (Table 2). In contrast, the five other samples with undetermined subgroups could not be recognized with either subgroup I- or subgroup II-specific MAbs (for example, samples 152, 184, and 186; Table 2). Two of them had long RNA patterns, but three specimens did not exhibit any RNA bands.

Serotype specificity could be determined in 58 (65.9%) samples: 18 were determined to be serotype 1, 17 were serotype 2, and 23 were serotype 4. No samples with serotype 3 specificity were detected. We observed an apparent yearly change in the serotype distributions between the two different collection years. Between 1983 and 1984, serotype 4 was detected with a high frequency, and between 1987 and 1988, serotype 2 was detected with a high fre-

TABLE 2. Reactivity patterns with MAbs of typical strains and strains with undetermined subgroup and serotype specificities

Stool specimen	ELISA results with[a]:								Ascribed:		RNA pattern
	YO-156 (common)	S2-37 (serogroup I)	YO-5 (serogroup II)	KU-6BG (serotype 1)	S2-2G10 (serotype 2)	YO-1E2 (serotype 3)	ST-2G7 (serotype 4)	YO-2C2 (common)	Subgroup	Serotype	
155	≥2,000	≥2,000	109	159	≥2,000	212	125	1,496	I	2	Short
250	1,209	67	662	1,634	112	82	99	578	II	1	Long
258	798	17	≥2,000	64	94	65	1,562	610	II	4	Long
122	918	665	403	236	177	96	131	244	I + II (?)	ND[b]	—[c]
124	896	406	678	201	138	116	112	343	I + II (?)	ND	—
152	1,270	110	132	126	148	85	71	185	ND	ND	—
184	1,680	113	99	85	38	21	34	242	ND	ND	Long
186	1,416	118	217	122	196	199	213	964	ND	ND	Long
231	≥2,000	150	≥2,000	107	157	81	71	504	II	ND	Long
240	≥2,000	64	≥2,000	108	153	68	83	479	II	ND	Long

[a] The data are shown as the optical density at 492 nm (in thousands) in a well. An optical density value of over 300 was considered to show a positive reaction. When the value for the reaction corresponding to a given serotype was greater than 2.0 times the value for the reactions corresponding to any other serotypes, the sample was assigned to a specific serotype. When the value for the reaction corresponding to a given subgroup was greater than 2.5 times the value for the reaction corresponding to the other subgroup, the sample was assigned to the specific subgroup.

[b] ND, Not determined.

[c] —, Enough RNA for RNA pattern analysis could not be obtained.

TABLE 3. ELISA results of porcine rotavirus strains with serotype 3 specificity

Fecal specimen	ELISA results with[a]:				
	KU-6BG (serotype 1)	S2-2G10 (serotype 2)	YO-1E2 (serotype 3)	ST-2G7 (serotype 4)	YO-2C2 (common)
21A	163	264	1,516	260	1,100
22A	261	209	1,506	206	1,238
37A	160	193	496	64	516
38A	138	149	409	62	500

[a] The ELISA was carried out by using MAbs as a capture antibody. The data are shown as the optical density at 492 nm (× 1,000) in a well.

quency (Table 1). The serotypes of 30 samples could not be determined. Of these, 28 samples did not react with any of four serotype-specific N-MAbs (for example, samples 231 and 240; Table 2). Judged from electron microscopic observations and reactivities with the commonly reactive YO-2C2 antibody recognizing double-shelled particles, 12 samples did not appear to contain enough double-shelled particles. However, 16 samples were reactive with the YO-2C2 antibody, and they had enough double-shelled particles (about 10 particles per electron microscope grid square). A total of 22 of the samples with undetermined serotypes were positive for RNA pattern analysis; most (19 samples) showed long RNA profiles, and 3 samples showed short RNA profiles.

Subgroup and serotype distributions of bovine and porcine rotaviruses in Thailand. A total of 25 bovine and 13 porcine specimens positive for group A rotavirus were tested for their subgroup and serotype specificities. All the bovine and porcine rotaviruses were subgroup I. One bovine strain did not react with the YO-156 antibody common to group A rotaviruses, but the specimen was strongly reactive with subgroup I-specific MAb. The specimen may have had a variation at the epitope that the YO-156 antibody recognizes, as has been suggested previously (23). Since we used only serotype 1- to 4-specific N-MAbs, because serotype 5- and 6-specific N-MAbs were not available, the serotype specificities of only seven porcine rotaviruses could be determined, although most of them reacted strongly with anti-VP4 cross-reactive YO-2C2 antibody. The seven porcine rotaviruses reacted significantly with serotype 3-specific MAb (Table 3). The RNA patterns of the 38 animal strains were analyzed by polyacrylamide gel electrophoresis. They all showed long RNA profiles, except for two porcine and two bovine specimens that were negative for RNA pattern analysis. One bovine rotavirus had an extra band between segments 9 and 10. Five serotype 3 porcine viruses had two or three extra bands between segments 4 and 5 that might have resulted from rearrangement of the RNA segments (Fig. 1).

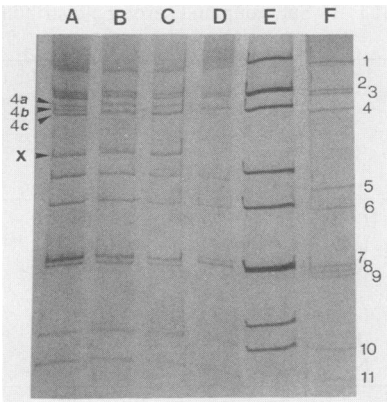

FIG. 1. Polyacrylamide gel electrophoresis of type 3 porcine rotavirus RNA genomes extracted from fecal specimens. Lanes: A, Porcine rotavirus 18A; B, porcine rotavirus 19A; C, porcine rotavirus 20A; D, porcine rotavirus 21A. Human subgroup I (lane E) and subgroup II (lane F) rotavirus RNAs from stool specimens were included as controls. Migration was from top to bottom. Numbers on the right designate RNA segments. Extra bands are indicated by arrowheads on the left.

DISCUSSION

Several studies on serotype distribution have been performed (3, 9, 10) since an ELISA with serotype-specific N-MAbs (ELISA serotyping) was developed. Serotype 1 appears to be the most prevalent serotype, but the serotype distribution is quite variable in different countries and within regions of countries. In this study, of the specimens for which the serotypes were determined, 40% were serotype 4, 31% were serotype 1, and 29% were serotype 2. However, distinct variations in the frequency of each serotype were found in different years, as was found in the study done with stool samples from humans in Venezuela (9). It was also notable that no virus with serotype 3 specificity was detected in any years of the investigation, although the number examined was relatively small. It is possible that the serotype 3-specific MAb used in this study had too narrow a reactivity spectrum. However, we can exclude this possibility because we confirmed the strong reactivities of the serotype 3-specific MAbs with representative type 3 rotavirus strains and because we detected serotype 3 at a high frequency in different studies by using the same MAb that we used in this study (9; Urasawa et al., in press).

Most bovine rotavirus strains have been grouped into serotype 6, which is represented by the prototype strains NCDV and UK (33). More bovine serotypes have been detected in rotaviruses from calves, but they have not yet been classified systematically. In this study, using MAbs specific to serotypes 1 to 4, we could not detect any bovine strains that shared a neutralization antigen with HRV.

In porcine rotavirus, serotypes 4 and 5, represented by the Gottfried and OSU strains, respectively, have been identified (2). In this study, we detected serotype 3 porcine rotavirus strains at a high frequency, although their failure to react with serotype 5-specific MAb should also be confirmed. In Australia, recently, two porcine rotaviruses (strains MDR-13 and CRW-8) that were antigenically related to human serotype 3 were isolated (20). These findings suggest that serotype 3 porcine rotaviruses are distributed worldwide. By implication, serotype 3 includes rotaviruses from various animal species, for example, humans, monkeys, cats, horses, rabbits, and pigs (8, 14, 15). Furthermore, in the murine model only HRV serotype 3 can cause diarrhea (1). Study of the possible transmission of rotavirus among different animal species in nature is required so that the ecology and evolution of rotaviruses can be considered. The serotype 3 porcine rotaviruses found in this study had two or three additional RNA bands in five strains. They do not seem to be due simply to a mixture of different strains, since the X band out of the extra bands (Fig. 1) had an unusual mobility that was not found in other group A rotaviruses. Rather, the appearance of the extra bands might be the result of a rearrangement of viral RNA, which has been observed especially in immunodeficient infants (22). Further studies

after cultivation of the viruses in cell culture are needed for elucidating this point.

Recent studies detected several group A rotavirus strains which had unusual antigenic specificities, for example, an equine strain with subgroup I and II specificities (12), a human strain with subgroup I specificity but a long RNA pattern (21), and human strains with new serotype specificities other than for serotypes 1 to 4 (6, 19). The simultaneous use of ELISA subgrouping, ELISA serotyping, RNA electropherotyping, and electron microscopy is useful for finding antigenic variants and strains with new serotypes or subgroups (7, 12, 25, 32). Some of the strains found in this study that could not be recognized by any serotype-specific MAbs (Table 2) might be grouped as new serotype(s) inclusive of human serotypes 5 and 6. Alternatively, they might have a variation at the position at which the MAbs used in this study were directed, and they might escape recognition by the MAbs. We must wait for the results of further serological analyses and molecular studies, such as nucleotide sequence analysis, before we can finally conclude whether they indeed have some unusual properties.

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LITERATURE CITED

1. **Bell, L. M., H. F. Clark, E. A. O'Brien, M. J. Kornstein, S. A. Plotkin, and P. A. Offit.** 1987. Gastroenteritis caused by human rotaviruses (serotype 3) in a suckling mouse model. Proc. Soc. Exp. Biol. Med. **184**:127–132.
2. **Bohl, E. H., K. W. Theil, and L. J. Saif.** 1984. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol. **19**:105–111.
3. **Brown, D. W. G., M. M. Mathan, M. Mathew, R. Martin, G. M. Beards, and V. I. Mathan.** 1988. Rotavirus epidemiology in Vellore, South India: group, subgroup, serotype, and electropherotype. J. Clin. Microbiol. **26**:2410–2414.
4. **Chiba, S., T. Yokoyama, S. Nakata, Y. Morita, T. Urasawa, K. Taniguchi, S. Urasawa, and T. Nakao.** 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. Lancet **ii**:417–421.
5. **Clark, H. F., F. E. Borian, L. M. Bell, K. Modesto, V. Gouvea, and S. A. Plotkin.** 1989. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J. Infect. Dis. **158**:570–587.
6. **Clark, H. F., Y. Hoshino, L. M. Bell, J. Groff, G. Hess, P. Bachman, and P. A. Offit.** 1987. Rotavirus isolate WI61 representing a presumptive new human serotype. J. Clin. Microbiol. **25**:1757–1762.
7. **Coulson, B. S.** 1987. Variation in neutralising epitopes of human rotaviruses in relation to genomic RNA polymorphism. Virology **159**:209–216.
8. **Estes, M., E. L. Palmer, and J. F. Objeski.** 1983. Rotaviruses: a review. Curr. Top. Microbiol. Immunol. **105**:123–184.
9. **Flores, J., K. Taniguchi, K. Green, I. Perez-Schael, D. Garcia, J. Sears, S. Urasawa, and A. Z. Kapikian.** 1988. Relative frequencies of rotavirus serotypes 1, 2, 3, and 4 in Venezuelan infants with gastroenteritis. J. Clin. Microbiol. **26**:2092–2095.
10. **Georges-Courbot, M. C., A. M. Beraud, G. M. Beards, A. D. Campbell, J. P. Gonzalez, A. J. Georges, and T. H. Flewett.** 1988. Subgroups, serotypes, and electropherotypes of rotavirus isolated from children in Bangui, Central African Republic. J. Clin. Microbiol. **26**:668–671.
11. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol. **19**:439–441.
12. **Hoshino, Y., M. Gorziglia, J. Valdesuso, J. Askaa, R. I. Glass, and A. Z. Kapikian.** 1987. An equine rotavirus (FI-14 strain) which bears both subgroup I and II specificities on its VP6. Virology **157**:488–496.
13. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**:8701–8704.
14. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotype similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque reduction neutralization. J. Infect. Dis. **149**:694–702.
15. **Kapikian, A. Z., and R. M. Chanock.** 1985. Rotaviruses, p. 863–906. *In* B. N. Fields, D. N. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (ed.), Virology. Raven Press, New York.
16. **Kapikian, A. Z., J. Flores, K. Midthun, Y. Hoshino, K. Y. Green, M. Gorziglia, K. Taniguchi, K. Nishikawa, R. M. Chanock, L. Potash, I. Perez-Schael, R. Doin, C. Chrysty, M. Santosham, N. A. Halsey, M. L. Clements, S. D. Sears, R. E. Black, M. M. Levine, G. Losonsky, M. Rennels, L. Gottefors, G. Wadell, R. I. Glass, T. Vesikari, E. L. Anderson, R. B. Belshe, P. F. Wright, and S. Urasawa.** 1988. Development of a rotavirus vaccine by a "Jennerian" and a modified "Jennerian" approach, p. 151–159. *In* H. Ginsberg, F. Brown, R. A. Lerner, and R. M. Chanock (ed.), Vaccines 88. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Lambert, J.-P., P. Marbehant, D. Marissens, and G. Zissis.** 1984. Monoclonal antibodies directed against different antigenic determinants of rotavirus. J. Virol. **51**:47–51.
18. **Liu, M., P. A. Offit, and M. K. Estes.** 1988. Identification of the simian rotavirus SA11 genome segment 3 product. Virology **163**:26–32.
19. **Matsuno, S., A. Hasegawa, A. Mukoyama, and S. Inouye.** 1985. A candidate for a new serotype of human rotavirus. J. Virol. **54**:623–624.
20. **Nagasha, H. S., and I. H. Holmes.** 1988. New porcine rotavirus serotype antigenicity related to human rotavirus serotype 3. J. Clin. Microbiol. **26**:171–174.
21. **Nakagomi, O., T. Nakagomi, Y. Hoshino, J. Flores, and A. Z. Kapikian.** 1987. Genetic analysis of a human rotavirus that belongs to subgroup I but has an RNA pattern typical of subgroup II human rotaviruses. J. Clin. Microbiol. **25**:1159–1164.
22. **Pedley, S., F. Hundley, I. Chrystie, M. A. McCrae, and U. Desselberger.** 1984. The genomes of rotaviruses isolated from chronically infected immunodeficient children. J. Gen. Virol. **65**:1141–1150.
23. **Pothier, P., E. Kohli, E. Drouet, and S. Ghim.** 1987. Analysis of the antigenic sites on the major inner capsid protein (VP6) of rotaviruses using monoclonal antibodies. Ann. Virol. (Paris) **138**:285–295.
24. **Ruiz, A. M., I. V. Lopez, S. Lopez, R. T. Espejo, and C. F. Arias.** 1988. Molecular and antigenic characterization of porcine rotavirus YM, a possible new rotavirus serotype. **62**:4331–4336.
25. **Svensson, L., L. Grahnquist, C. Petterson, M. Grandian, G. Stintzing, and H. B. Greenberg.** 1988. Detection of human rotaviruses which do not react with subgroup I- and II-specific monoclonal antibodies. J. Clin. Microbiol. **26**:1238–1240.
26. **Taniguchi, K., Y. Morita, T. Urasawa, and S. Urasawa.** 1987. Cross-reactive neutralization epitopes on VP3 of human rotavirus: analysis with monoclonal antibodies and antigenic variants. J. Virol. **61**:1726–1730.
27. **Taniguchi, K., T. Urasawa, Y. Morita, H. B. Greenberg, and S. Urasawa.** 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. **155**:1159–1166.
28. **Taniguchi, K., S. Urasawa, and T. Urasawa.** 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity patterns to human rotaviruses. J. Gen. Virol. **66**:1025–1035.
29. **Taniguchi, K., T. Urasawa, S. Urasawa, and T. Yasuhara.** 1984. Production of subgroup-specific monoclonal antibodies against human rotaviruses and their application to an enzyme-linked

immunosorbent assay for subgroup determination. J. Med. Virol. **14**:115–125.

30. **Urasawa, T., S. Urasawa, K. Taniguchi, and S. Chiba.** 1984. Serotype determination of human rotavirus isolates and antibody prevalence in pediatric population in Hokkaido, Japan. Arch. Virol. **81**:1–12.
31. **Urasawa, S., T. Urasawa, K. Taniguchi, Y. Morita, N. Sakurada, Y. Saeki, O. Morita, and S. Hasegawa.** 1988. Validity of an enzyme-linked immunosorbent assay with serotype-specific monoclonal antibodies for serotyping human rotavirus in stool specimens. Microbiol. Immunol. **32**:699–708.
32. **Urasawa, T., S. Urasawa, Y. Chiba, K. Taniguchi, N. Kobayashi, L. N. Mutanda, and P. M. Tukei.** 1987. Antigenic characterization of rotavirus isolated in Kenya from 1982 to 1983. J. Clin. Microbiol. **25**:1891–1896.
33. **Woode, G. N., S. Zheng, B. I. Rosen, N. Knight, N. E. Kelso Gourley, and R. F. Ramig.** 1987. Protection between different serotypes of bovine rotavirus in gnotobiotic calves: specificity of serum antibody and coproantibody responses. J. Clin. Microbiol. **25**:1052–1058.