

Survey of Human Group C Rotaviruses in Japan during the Winter of 1992 to 1993

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Fecal specimens from patients with acute diarrhea were collected from 10 prefectures in Japan over a 6-month period (November 1992 to April 1993), and the specimens that were negative for human group A rotaviruses were screened for the presence of human group C rotaviruses (CHRVs) by the reverse passive hemagglutination test. Of 784 specimens examined, 53 samples (6.8%) that were collected in 7 of 10 prefectures were positive for CHRV, indicating that CHRVs are widely distributed across Japan. Most of the CHRV isolates were detected in March and April, and CHRVs mainly prevailed in children ages 3 to 8 years. The genome electropherotypes of eight strains isolated in five individual prefectures were surprisingly similar to each other and were different from those of CHRV strains isolated to date. The outer capsid glycoprotein (VP7) gene homologies of the isolates retrieved in 1993 were subsequently analyzed by the dot blot hybridization method. As a result, the VP7 genes of the isolates revealed very high levels of homology not only with each other but also with the VP7 gene of the OK118 strain isolated in 1988. These results suggest that a large-scale outbreak of CHRV occurred during the winter of 1992 and 1993 in Japan.

Rotaviruses are recognized as the major etiologic agents of diarrheal diseases in young children and animals. The genome of rotaviruses consists of 11 segments of double-stranded RNA (dsRNA) enclosed in a double-shelled particle. Rotaviruses are classified into seven groups (groups A to G) on the basis of their dsRNA electropherotypes and a common group antigen on the inner capsid protein (protein VP6) (19).

Group C rotaviruses were first recognized in swine (2, 20) and then were confirmed as human pathogens by Bridger et al. (4). During the last decade, human group C rotaviruses (CHRVs) have been associated with several outbreaks of acute diarrhea in Asia (12, 18), Europe (3, 5), and South America (7). More recently, CHRVs have been detected in patients with sporadic cases of diarrhea in the United States (9). These observations indicate that CHRV is widely distributed and is likely to be an emerging pathogen.

CHRV infections in Japan were first recognized by Oseto et al. (17) in 1985. Ushijima et al. (23) also detected CHRVs from fecal specimens collected in the Tokyo area in 1987. Since then, some researchers have recognized CHRV infections in individual locations (6, 14, 16). However, none of these investigators have carried out an epidemiological study that covers several locations in Japan.

Until recently, the detection of CHRVs in fecal specimens was usually performed by immune electron microscopy with

CHRV-specific antisera or polyacrylamide gel electrophoresis (PAGE) of viral dsRNA, because CHRVs were noncultivable. The difficulty in detection has hindered the epidemiological analysis of CHRV infections. Recently, we have developed a reverse passive hemagglutination (RPHA) test using CHRV-specific monoclonal antibodies (11). This test is very easy to perform and should be a useful tool for the screening of CHRVs on a large scale.

In this study, we collected fecal specimens from patients with diarrhea in 10 prefectures in Japan and examined the specimens for the presence of CHRVs by the RPHA test. As a result, CHRVs were detected in seven prefectures. To define the genetic relationship among the CHRV field isolates, we analyzed the genome electropherotypes and the outer capsid glycoprotein (VP7) gene homologies of the isolates.

MATERIALS AND METHODS

Fecal specimens. Between November 1992 and April 1993, 1,114 fecal specimens from patients with acute diarrhea were collected at pediatric clinics or outpatient sections of general hospitals in 10 prefectures in Japan. Figure 1 shows the geographic areas in which the specimens were collected.

All fecal specimens were screened for human group A rotaviruses with an enzyme-linked immunosorbent assay (ELISA) kit (ROTAclone; Cambridge Biotech, Worcester, Mass.). The specimens that were negative for human group A rotaviruses were further examined for the presence of CHRV by the RPHA test.

RPHA test. The RPHA test was performed as described previously (11). Briefly, 10% suspensions of the fecal specimens in phosphate-buffered saline (pH 7.2) were centrifuged at $2,000 \times g$ for 10 min and the supernatants were tested. Serial twofold dilutions of the samples were made in duplicate. In one dilution series, a 0.7% suspension of sheep erythrocytes (SRBCs) coated with CHRV-specific monoclonal antibodies was added to each well. In the other series, SRBCs coated with normal mouse immunoglobulin G (control SRBCs) were

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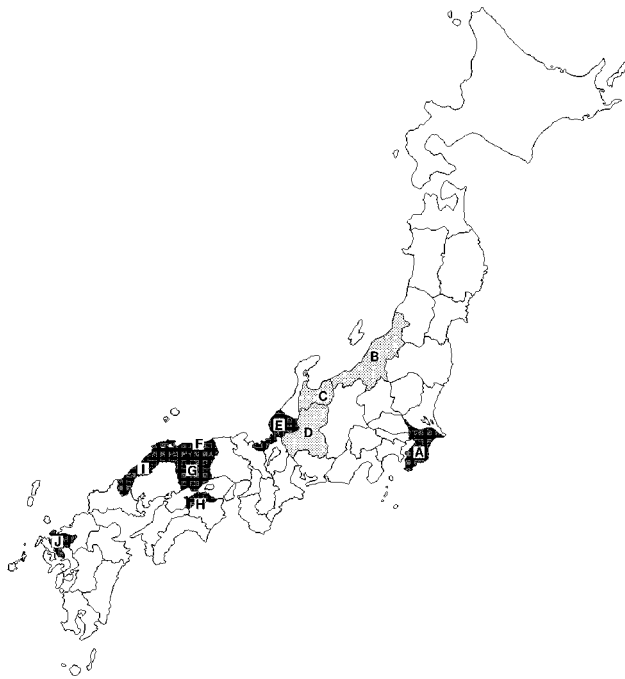


FIG. 1. Map of Japan showing the prefectures from which the fecal specimens were collected. Darker shading indicates the prefectures in which CHRVs have been detected. A, Chiba; B, Niigata; C, Toyama; D, Gifu; E, Fukui; F, Tottori; G, Okayama; H, Kagawa; I, Shimane; J, Saga.

added. Hemagglutination titers were observed after 1 h. When the RPHA test titer with monoclonal antibody-coated SRBCs was four or more times greater than that obtained with the control SRBCs, the sample was judged to be CHR V positive.

RNA extraction. Fecal suspensions containing CHR Vs were extracted with an equal volume of trichlorotrifluoroethane, and the supernatants were adjusted to contain 10 mM EDTA, 0.6% sodium dodecyl sulfate (SDS), and 300 µg of proteinase K per ml. The suspensions were incubated for 1.5 h at 40°C and then extracted with phenol-chloroform. Viral RNA was further purified with an RNAID kit (Bio 101, Inc., La Jolla, Calif.) according to the manufacturer's instructions. The purified RNAs were stored at -30°C until use.

Dot blot hybridization. Hybridization tests were carried out as described previously (10). In brief, the VP7 genes of CHR V isolates were first amplified by the reverse transcription-PCR (RT-PCR) method (10) and were then purified with a Suprec-02 column (Takara Shuzo Co., Ltd., Kyoto, Japan). Equivalent amounts (200 ng) of the genes amplified by RT-PCR were blotted onto nylon

membranes and were separately hybridized with K9304, OK118, and OK450 VP7 genes that had been labeled with digoxigenin-11-dUTP (Boehringer, Mannheim, Germany). Hybridization was performed under highly stringent conditions (50% formamide and 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] at 52°C). After hybridization, the membranes were washed twice in 0.1× SSC containing 0.1% SDS at 68°C for 15 min. Colorimetric detection of the hybridized probe was carried out according to the manufacturer's instructions.

Sequencing of the VP7 genes. The VP7 gene of a clinical isolate (isolate K9304) was amplified by the RT-PCR method and was then cloned into plasmid pUC18 (10). Five individual recombinants were isolated, and both strands of the cloned DNA were sequenced by the dideoxynucleotide chain-termination method (Applied Biosystems, Foster City, Calif.). Nucleotide sequence data were then analyzed by the GENETYX-MAC, version 6.0, program. The program MAlign, version 1.0, was also used for sequence alignments.

Nucleotide sequence accession number. The nucleotide sequence data for the VP7 gene from strain K9304 has been submitted to the DDBJ DNA database and has been assigned accession number AB004250.

RESULTS

Detection of CHR Vs from fecal specimens by RPHA test.

The results of the detection of rotaviruses are summarized in Table 1. Human group A rotaviruses were detected in 330 of 1,114 specimens. Group A rotavirus-negative samples were further examined for CHR Vs by the RPHA test, and 53 were positive. The geographic distribution of the seven prefectures in which CHR Vs have been detected is shown in Fig. 1. CHR Vs were mainly distributed in the western area of Japan. The rates of positivity for CHR V were considerably lower than those for human group A rotavirus (Table 1). No significant difference was observed between the rates of positivity for males and females (data not shown).

The epidemiological features of CHR V infections were then compared with those of human group A rotavirus infections. CHR Vs were mainly detected in March and April (Table 1), whereas most human group A rotavirus infections occurred between January and February (data not shown). The age-specific attack rates for human group C and group A rotaviruses are compared in Table 2. Although CHR Vs principally prevailed in children ages 3 to 8 years, the target age groups of human group A rotavirus were below 3 years. The mean age of the patients infected with CHR V (4.36 years) was significantly ($P < 0.01$) higher than that of patients infected with human group A rotavirus (2.19 years).

Genome electropherotypes of CHR V field isolates. Only eight specimens which were obtained from five prefectures had enough volume from which viral dsRNA could be extracted for genome electropherotype analyses. The RNA segments were

TABLE 1. Detection of rotaviruses in 10 prefectures

Prefecture	Detection result ^a		No. of CHR V isolates detected in at the following times:						
	Human group A rotaviruses ^b	CHR Vs ^c	1992		1993				
			November	December	January	February	March	April	
Chiba	24/97 (24.7)	2/73 (2.7)						1	1
Niigata	7/55 (12.7)	0/48							
Toyama	49/119 (41.2)	0/70							
Gifu	20/74 (27)	0/54							
Fukui	6/21 (28.6)	2/15 (13.3)							2
Tottori	46/123 (37.4)	6/77 (7.8)							6
Okayama	22/176 (12.5)	11/154 (7.1)		2			2	3	4
Kagawa	83/163 (50.9)	8/80 (10)		2	1	1	1	3	1
Shimane	30/187 (16)	19/157 (12.1)	1		4	6	6	6	2
Saga	43/99 (43.4)	5/56 (8.9)			2	2	2	1	
Total	330/1,114 (29.6)	53/784 (6.8)	1	4	7	11	14	14	16

^a Values indicate number of samples that were positive/total number of samples tested. Values in parentheses indicate percentage of tests that were positive.

^b Human group A rotaviruses were detected with a commercially available ELISA kit.

^c CHR Vs were detected by the RPHA test.

TABLE 2. Comparison of age-specific attack rates for CHR V and human group A rotavirus

Age group (yr)	Attack rate (%)	
	CHR V	Human group A rotavirus
<1	4.2	38.7
1-2	8.9	42.3
3-4	12.8	24.2
5-6	6.2	14.5
7-8	10.0	10.7
9-10	4.2	4.0
>10	2.6	10.0

dissociated by PAGE with a 10% gel and were then visualized with silver nitrate. Figure 2 (lanes 1 to 8) shows the genome electropherotypes of the field isolates. Every strain exhibited the typical 4-3-2-2 profile of group C rotavirus. Although the strains were isolated in five individual prefectures, the electropherotypes of all strains were similar to each other.

We previously demonstrated that the genome electropherotypes of CHR Vs isolated in Okayama from 1988 to 1990 were classified into patterns I and II (6, 10). The electropherotype of the K9304 strain, which was selected as a representative of the isolates retrieved in 1993, was then compared with those of the OK118 (pattern I) and the OK450 (pattern II) strains in the same gel (Fig. 2, lanes 9 to 11). The RNA profile of K9304 was different from those of both OK118 and OK450, and so this electropherotype was tentatively designated pattern III. The electrophoretic mobilities of the 2nd, 3rd, 7th, 10th, and 11th segments of K9304 were similar to those of the corresponding segments of OK118, while the 1st, 4th, 5th, and 6th segments migrated to the same positions as those of OK450, suggesting that pattern III is a combined electropherotype of patterns I and II.

Analysis of the VP7 genes from CHR V field isolates. The VP7 gene homologies of the field isolates were further analyzed by dot blot hybridization with VP7 gene probes from the

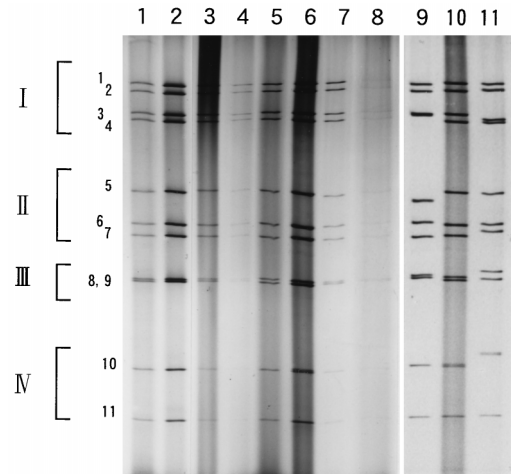


FIG. 2. Comparison of genome electropherotypes of CHR V field isolates. Lanes: 1, 93K332 strain (Chiba isolate); 2, 93K360 strain (Chiba isolate); 3, F522 strain (Fukui isolate); 4, 34803 strain (Tottori isolate); 5, T360 strain (Okayama isolate); 6, K9304 strain (Okayama isolate); 7, 93-48 strain (Saga isolate); 8, 93-42 strain (Saga isolate); 9, OK118 strain, which exhibits the pattern I electropherotype; 10, K9304 strain; 11, OK450 strain, which exhibits the pattern II electropherotype.

K9304, OK118, and OK450 strains. Epidemiological data and the hybridization results for the 19 isolates used in this study are presented in Table 3. The VP7 genes of all isolates strongly reacted with the K9304 probe as well as the OK118 probe, while weak hybridization signals were observed with the OK450 probe. These results indicate that the VP7 genes of the isolates retrieved in 1993 have high levels of homology not only with each other but also with the VP7 gene of the OK118 strain, which was isolated in 1988.

To confirm our findings, the VP7 gene of K9304 was cloned and sequenced. The VP7 gene of K9304 was 1,063 nucleotides in length and contained single open reading frame encoding 332 amino acids. The nucleotide and deduced amino acid se-

TABLE 3. Epidemiological data, genome patterns, and dot blot hybridization results for 19 CHR V isolates

Strain	Date of isolation (mo/day/yr)	Age (yr)	Sex ^a	Prefecture	Genome pattern	Dot blot result with the following probe:		
						K9304	OK118	OK450
OK118	2/6/1988	6	M	Okayama	I	+	+	-
OK450	2/8/1989	1	F	Okayama	II	-	-	+
93K332	3/23/1993	24	F	Chiba	III	+	+	-
93K360	4/27/1993	9	M	Chiba	III	+	+	-
F522	4/30/1993	1	M	Fukui	III	+	+	-
F618	4/24/1993	4	F	Fukui	ND ^b	+	+	-
34745	4/23/1993	15	M	Tottori	ND	+	+	-
34803	4/12/1993	1	F	Tottori	III	+	+	-
T360	3/6/1993	3	M	Okayama	III	+	+	-
K12	3/15/1993	6	M	Okayama	ND	+	+	-
K9301	4/19/1993	8	M	Okayama	ND	+	+	-
K9304	4/22/1993	4	M	Okayama	III	+	+	-
K9306	4/26/1993	4	F	Okayama	ND	+	+	-
KA63	3/3/1993	7	M	Kagawa	ND	+	+	-
KA73	3/30/1993	5	M	Kagawa	ND	+	+	-
S373	1/25/1993	1	F	Shimane	ND	+	+	-
S891	2/2/1993	1	M	Shimane	ND	+	+	-
93-42	2/2/1993	1	M	Saga	III	+	+	-
93-48	2/23/1993	1	M	Saga	III	+	+	-

^a M, male; F, female.

^b ND, genome pattern could not be determined.

TABLE 4. Nucleotide and deduced amino acid sequence homologies of VP7 genes from three CHRv strains

Strain	% Homology to the following strain ^a :			Accession no.
	K9304	OK118	OK450	
K9304		99.2	95.6	AB004250
OK118	99.4		95.7	D87543
OK450	96.7	96.7		D87544

^a The percent homologies of nucleotide sequences are given in boldface. Other data represent the percent homologies of deduced amino acid sequences.

quences of the VP7 gene from K9304 were further compared with those of the genes from OK118 and OK450. As indicated in Table 4, a surprising level of sequence conservation was observed between K9304 and OK118 (more than 99.1%), whereas the overall nucleotide and amino acid identities between K9304 and OK450 were relatively low (95.6 and 96.7%, respectively).

DISCUSSION

This study, the first survey of CHRVs in various locations in Japan, indicated that CHRVs are widely distributed in Japan. The rates of CHRv positivity ranged from 2.7 to 13.3%, and the CHRv isolates were mainly detected in March and April. Moreover, CHRVs principally prevailed in children ages 3 to 8 years. These epidemiological features are clearly distinct from those of the human group A rotavirus. Oseto (16) previously carried out an epidemiological study of CHRVs over a 3-year period in Matsuyama City, Japan. Our observations were consistent with those reported by Oseto (16).

In this study, we screened only the human group A rotavirus-negative specimens for the presence of CHRv. Jiang et al. (9) have recently reported mixed infections with human group A and group C rotaviruses in the United States. We therefore examined by the RPHA test group A rotavirus-positive specimens ($n = 52$) that were collected in Okayama and Shimane (data not shown), but none of the specimens was positive for CHRv, indicating that the mixed infection might be rather rare. In fact, Jiang et al. (9) recognized only one mixed infection among 1,676 samples. However, screening of rotavirus infections must hereafter include screening for mixed infection.

The RPHA test could successfully detect CHRVs even in fecal specimens that were insufficient for immune electron microscopy or genome electropherotype analyses. To inspect the specificity of the RPHA test, the RPHA test-positive specimens were examined by our ELISA system (6), and all were determined to be positive. Recently, the PCR method has been applied to the detection of group C rotaviruses by Gouvea et al. (8). Although the sensitivity of the RPHA test is not comparable to that of the PCR method, the former test is faster and simpler and is more suitable for use for routine diagnosis in clinical settings.

In group A rotaviruses, genome electropherotyping of field isolates is a useful tool for obtaining epidemiological information about the origin of the isolates and diversity among these isolates, because each isolate reveals a unique genome profile (1, 22). The genome electropherotypes of the isolates retrieved in 1993 were surprisingly similar to each other, regardless of the prefectures from which the isolates were obtained. Moreover, the dot blot hybridization analysis showed that the VP7 genes of the isolates were highly homologous. These results strongly suggest that a large-scale outbreak of CHRv occurred

during the winter of 1992 and 1993 in Japan. However, further comparative analysis of other genome segments will be required to confirm this hypothesis.

The electropherotype of the K9304 strain, which represented isolates retrieved in 1993, was compared with those of the pattern I and II strains in the same gel. Although K9304 revealed a distinct genome profile tentatively designated pattern III, this electropherotype seemed to be a combination of patterns I and II. The sequence analysis of the VP7 gene from K9304 also showed that the VP7 gene of K9304 was similar to that of the pattern I strain. These results indicate that the K9304 strain may be a reassortant virus between the pattern I and pattern II strains, because it has been reported that natural reassortants occurred between human group A rotaviruses strains belonging to different genogroups (13, 24). Quite recently, CHRVs have been successfully propagated in a continuous cell line (CaCo-2) (15, 21). To clarify the genetic and antigenic relationship among three strains with distinct electropherotypes, we are now attempting to adapt these strains to CaCo-2 cells.

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REFERENCES

- Ahmed, M. U., S. Urasawa, K. Taniguchi, T. Urasawa, N. Kobayashi, F. Wakasugi, A. I. M. M. Islam, and H. A. Sahikh. 1991. Analysis of human rotavirus strains prevailing in Bangladesh in relation to nationwide floods brought by the 1988 monsoon. *J. Clin. Microbiol.* **29**:2273-2279.
- Bohl, E. H., L. J. Saif, K. W. Theil, A. G. Agnes, and R. F. Cross. 1982. Porcine parrotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. *J. Clin. Microbiol.* **15**:312-319.
- Bonsdorf, C. H. V., and L. Svensson. 1988. Human serogroup C rotavirus in Finland. *Scand. J. Infect. Dis.* **20**:475-478.
- Bridger, J. C., S. Pedley, and M. A. McCrae. 1986. Group C rotaviruses in humans. *J. Clin. Microbiol.* **23**:760-763.
- Caul, E. O., C. R. Ashley, J. M. Darville, and J. C. Bridger. 1990. Group C rotavirus associated with fatal enteritis in a family outbreak. *J. Med. Virol.* **30**:201-205.
- Fujii, R., M. Kuzuya, M. Hamano, M. Yamada, and S. Yamazaki. 1992. Detection of human group C rotaviruses by an enzyme-linked immunosorbent assay using monoclonal antibodies. *J. Clin. Microbiol.* **30**:1307-1311.
- Gabbay, Y. B., J. D'Arc, P. Mascarenas, A. C. Linhares, and R. B. Freitas. 1989. Atypical rotavirus among diarrhoeic children living in Belém, Brazil. *Mem. Inst. Oswaldo Cruz* **84**:5-7.
- Gouvea, V., J. R. Allen, R. I. Glass, Z.-Y. Fang, M. Bremont, J. Cohen, M. A. McCrae, L. J. Saif, P. Sinarachatanant, and E. O. Caul. 1991. Detection of group B and C rotaviruses by polymerase chain reaction. *J. Clin. Microbiol.* **29**:519-523.
- Jiang, B. M., P. H. Dennehy, S. Spangenberg, J. R. Gentsch, and R. I. Glass. 1995. First detection of group C rotavirus in fecal specimens of children with diarrhea in the United States. *J. Infect. Dis.* **172**:45-50.
- Kuzuya, M., R. Fujii, M. Hamano, J. Nakamura, M. Yamada, S. Nii, and T. Mori. 1996. Molecular analysis of outer capsid glycoprotein (VP7) genes from two isolates of human group C rotavirus with different genome electropherotypes. *J. Clin. Microbiol.* **34**:3185-3189.
- Kuzuya, M., R. Fujii, M. Hamano, T. Nagabayashi, H. Tsunemitsu, M. Yamada, S. Nii, and T. Mori. 1993. Rapid detection of human group C rotaviruses by reverse passive hemagglutination and latex agglutination tests using monoclonal antibodies. *J. Clin. Microbiol.* **31**:1308-1311.
- Matsumoto, K., M. Hatano, K. Kobayashi, A. Hasegawa, S. Yamazaki, S. Nakata, S. Chiba, and Y. Kimura. 1989. An outbreak of gastroenteritis associated with acute rotaviral infection in schoolchildren. *J. Infect. Dis.* **160**:611-615.
- Nakagomi, O., and T. Nakagomi. 1991. Molecular evidence for naturally occurring single VP7 gene substitution reassortant between human rotavi-

- ruses belonging to two different genogroups. *Arch. Virol.* **119**:67–81.
14. **Oishi, I., K. Yamazaki, and Y. Minekawa.** 1993. An occurrence of diarrheal cases associated with group C rotavirus in adults. *Microbiol. Immunol.* **37**: 505–509.
 15. **Oseto, M., Y. Yamashita, M. Hattori, M. Mori, H. Inoue, Y. Ishimaru, and S. Matsuno.** 1994. Serial propagation of human group C rotavirus in a continuous cell line (CaCo-2). *J. Clin. Exp. Med.* **168**:177–178. (In Japanese.)
 16. **Oseto, M.** 1990. Epidemiological study of group C rotavirus. *J. Jpn. Assoc. Infect. Dis.* **64**:1264–1273. (In Japanese.)
 17. **Oseto, M., Y. Yamashita, M. Okuyama, H. Kuwabara, and H. Inoue.** 1986. Detection of atypical rotaviruses by polyacrylamide gel electrophoresis. *J. Clin. Exp. Med.* **136**:223–224. (In Japanese.)
 18. **Penaranda, M. E., W. D. Cubitt, P. Sinarachatanant, D. N. Taylor, S. Likanonsakul, L. Saif, and R. I. Glass.** 1989. Group C rotavirus infection in patients with diarrhea in Thailand, Nepal, and England. *J. Infect. Dis.* **160**: 392–397.
 19. **Saif, L. J.** 1990. Nongroup A rotaviruses, p. 73–95. *In* L. J. Saif and K. W. Theil (ed.), *Viral diarrhea of man and animals*. CRC Press, Inc., Boca Raton, Fla.
 20. **Saif, L. J., E. H. Bohl, K. W. Theil, R. F. Cross, and J. A. House.** 1980. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. *J. Clin. Microbiol.* **12**:105–111.
 21. **Shinozaki, K., T. Yamanaka, M. Tokieda, H. Shirasawa, and B. Simizu.** 1996. Isolation and serial propagation of human group C rotaviruses in a cell line (CaCo-2). *J. Med. Virol.* **48**:48–52.
 22. **Theil, K. W.** 1990. Group A rotaviruses, p. 35–72. *In* L. J. Saif and K. W. Theil (ed.), *Viral diarrhea of man and animals*. CRC Press, Inc., Boca Raton, Fla.
 23. **Ushijima, H., H. Honma, A. Mukoyama, T. Shinozaki, Y. Fujita, M. Kobayashi, M. Oseto, M. Morikawa, and T. Kitamura.** 1989. Detection of group C rotaviruses in Tokyo. *J. Med. Virol.* **27**:299–303.
 24. **Ward, R. L., O. Nakagomi, D. R. Knowlton, M. M. McNeal, T. Nakagomi, J. D. Clemens, D. A. Sack, and G. M. Schiff.** 1990. Evidence for natural reassortants of human rotaviruses belonging to different genogroups. *J. Virol.* **64**:3219–3225.