JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1992, p. 3009–3012
0095-1137/92/113009-04$02.00/0

Vol. 30, No. 11

Evidence of Serologic Diversity within Group C Rotaviruses†

HIROSHI TSUNEMITSU,[1,2] BAOMING JIANG,[2]‡ YASUTAKA YAMASHITA,[3] MITSUAKI OSETO,[3] HIROSHI USHIJIMA,[4] AND LINDA J. SAIF[2]*

Hokkaido Prefectural Shintoku Animal Husbandry Experiment Station, Shintoku, Hokkaido 081,[1] Ehime Prefectural Institute of Public Health, 234-8 Sanban-cho, Matsuyama, Ehime 790,[3] and Department of Enteroviruses, National Institute of Health, Musashimurayama, Tokyo 208,[4] Japan, and Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio 44691[2]

Received 26 May 1992/Accepted 13 August 1992

The Cowden strain of porcine group C rotavirus and the Shintoku strain of bovine group C rotavirus were classified as different serotypes by two-way cross-neutralization tests. Two neutralization patterns against the Cowden and Shintoku strains were observed when hyperimmune or convalescent-phase antisera to three noncultivatable porcine group C rotaviruses and a human group C rotavirus were used in one-way cross-neutralization tests. Antisera to two porcine group C rotaviruses and the human group C rotavirus neutralized the Cowden strain at high titers but did not neutralize the Shintoku strain, suggesting that these three strains are serotypically related to the Cowden strain. The remaining antisera to a porcine group C rotavirus (HF strain) reacted with the Cowden and Shintoku group C rotaviruses in cell culture immunofluorescence tests but did not neutralize either virus in one-way cross-neutralization, suggesting that the HF strain belongs to a third serotype. However, confirmation of these findings requires additional analysis by two-way cross-neutralization. Our findings support the existence of at least two distinct serotypes of group C rotaviruses, and possibly a third, among animals and humans. The serotypic similarity observed between the Cowden strain and a human group C rotavirus suggests that the cultivatable Cowden strain and antiserum to this virus may provide important reagents for the diagnosis of group C rotaviruses in humans.

Rotaviruses are classified as a genus of the family *Reoviridae* on the basis of characteristic morphology and a genome containing 11 segments of double-stranded (ds) RNA. Rotaviruses are divided into seven groups (A to G) on the basis of their distinct antigenicity and dsRNA electropherotypes (18, 23, 24, 27, 31, 32).

The group (gp) A rotaviruses are a major cause of gastroenteritis in animal and human neonates (8, 17) and, compared with non-gp A rotaviruses, have been well studied and characterized. The gp C rotaviruses have been found in swine (2, 12, 29), humans (1, 5, 6, 16, 22, 25, 37), and cattle (36) with diarrhea. Porcine gp C rotaviruses cause diarrhea in experimentally inoculated gnotobiotic pigs (2, 30), and serologic surveys have indicated that gp C rotaviruses are prevalent in pigs in North America, Australia, Europe, and Japan (4, 19, 27, 35). Recently, a large-scale outbreak of diarrhea caused by gp C rotavirus in children in Japan has been reported (16), and others have also observed additional gp C rotavirus infections in humans (6, 22, 25, 37). These results suggest that gp C rotaviruses may be emerging enteric pathogens in animals and humans.

The gp A rotaviruses are classified into serotypes as defined by two outer capsid proteins (VP4 and VP7) which stimulate the production of neutralizing antibodies (10, 20). The predominant immune response in hyperimmunized animals is against VP7; therefore, neutralization assays for serotyping using hyperimmune antiserum reflect antigen properties of VP7 (7). Eleven serotypes of gp A rotaviruses have been identified on the basis of VP7 (7, 15). VP7 is encoded by gene segment 7, 8, or 9, depending on the virus strain (7). For gp C rotaviruses, an outer capsid glycoprotein corresponding to VP7 of gp A rotaviruses was identified (3, 14) and is encoded by gene 8 (26). Whether serotypic variation exists among gp C rotaviruses is unclear, and the failure of in vitro propagation of most gp C rotaviruses has hampered the use of neutralization assays for serotyping of gp C strains.

Recently, two strains of gp C rotaviruses (Cowden and Shintoku) have been successfully propagated in MA104 cells in our laboratory (30, 36). We also have analyzed the genetic diversity among gp C rotaviruses by DNA-RNA hybridization by using a gene 8 cDNA probe generated from the Cowden strain of porcine gp C rotavirus (13). But whether the genetic diversity noted in the hybridization assay actually reflects distinct antigenicities among gp C rotaviruses has not been determined by serologic assays. This report describes the serologic comparison of the Cowden and Shintoku strains of gp C rotaviruses by neutralization assays. Furthermore, the reactivities of these two gp C strains in neutralization assays with hyperimmune and convalescent-phase antisera against four noncultivated gp C rotaviruses are reported.

The Cowden porcine gp C and Shintoku bovine gp C rotaviruses adapted in MA104 cells (30, 36) were used as viral antigens for indirect immunofluorescence (IF) and fluorescent-focus neutralization (FFN) tests in this study. Both viruses were plaque purified at least once before use. Hyperimmune antisera were prepared in guinea pigs with gp C rotavirus strains Cowden, Shintoku, and Ehime 86-542 (human) and three porcine field strains designated WH, Wi, and HF (13). The viruses showed distinct dsRNA electropherotypes and were derived from intestinal contents of infected gnotobiotic pigs (Cowden, WH, Wi, and HF), tissue

* Corresponding author.

† Ohio State University journal article no. 158-92.

‡ Present address: Viral Gastroenteritis Unit, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA 30333.

TABLE 1. Indirect IF and neutralizing antibody titers of hyperimmune guinea pig antisera to four porcine rotaviruses, a bovine rotavirus, and a human group C rotavirus against Cowden porcine and Shintoku bovine group C rotaviruses

Antiserum to group C rotavirus (host-strain)	Indirect IF antibody titer[a] against rotavirus		Neutralizing antibody titer[b] against rotavirus	
	Porcine group C (Cowden)	Bovine group C (Shintoku)	Porcine group C (Cowden)	Bovine group C (Shintoku)
Porcine-Cowden	2,560	2,560	10,240	640
Bovine-Shintoku	10,240	10,240	640	51,200
Human-Ehime 86-542	1,280	1,280	2,560	80
Porcine-Wi	2,560	1,280	5,120	<80
Porcine-WH	5,120	2,560	5,120	<80
Porcine-HF	2,560	2,560	<80	<80

[a] Determined as the reciprocal of the highest antiserum dilution which resulted in fluorescing cells.
[b] Determined as the reciprocal of the highest antiserum dilution which caused an 80% or greater reduction in fluorescent foci.

culture fluids (Shintoku), or feces collected from a child with diarrhea (Ehime 86-542) in Ehime, Japan, and semipurified on sucrose gradients (14). Before injection of viruses into guinea pigs, the viruses were confirmed to be double shelled by electron microscopy and the guinea pigs were shown to be free of antibody to both gp C rotavirus strains by indirect IF and FFN tests. Hyperimmune antiserum against the Cowden strain was also prepared in gnotobiotic pigs as described previously (2). Seven gnotobiotic pigs and a colostrum-deprived calf were used for production of specific convalescent-phase antisera against Cowden, WH, Wi, and HF porcine gp C rotaviruses and Shintoku bovine gp C rotavirus, respectively. All pigs and the calf developed diarrhea at postinoculation days 1 to 3, and diarrhea continued for 2 to 6 days. Coincident with diarrhea, gp C rotavirus shedding detected by immune electron microscopy (28) and electrophoresis of viral dsRNA (34) occurred in feces from all pigs and the calf. At 18 to 21 days after inoculation, the pigs and calf were bled. Convalescent-phase sera were collected from four children in Ehime, Japan, naturally infected with a human gp C rotavirus which showed the same dsRNA electropherotype as the Ehime 86-542 strain (22).

Antibody titers of the antisera were determined by indirect IF tests as described previously (33, 36). The neutralization titers of antisera against the Cowden and Shintoku gp C rotavirus strains were determined by the FFN test as described previously (21). Briefly, equal volumes of sera (serial twofold dilutions) and viruses containing 200 fluorescence focus units per 0.05 ml were mixed and reacted at 37°C for 2 h. The mixture (0.05 ml) was inoculated onto monolayers of MA104 cells grown in 96-well plates. After adsorption at 37°C for 1 h, the cells were washed once with Eagle's minimum essential medium; they then received the same medium containing 25 μg of pancreatin per ml and were incubated at 37°C for 20 h. The cells were fixed with 80% acetone and stained with gnotobiotic pig anti-porcine gp C rotavirus (Cowden) serum conjugated with fluorescein isothiocyanate. Neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci. The initial serum dilution for the FFN test was determined by testing sera from 10 nonimmunized animals (five gnotobiotic pigs and five guinea pigs). Four sera (from one gnotobiotic pig and three guinea pigs) showed nonspecific background reactions to the Cowden or Shintoku strain up to a dilution of 1:40. Therefore, the initial serum dilution used for the FFN test was 1:80. The reproducibility of both tests was evaluated by testing five sera on three separate occasions. There was no greater than a twofold variation in the titer of each serum in both tests.

The indirect IF and neutralizing antibody titers of various hyperimmune guinea pig antisera against the Cowden and Shintoku strains are shown in Table 1. All of the antisera possessed indirect IF antibody to both strains at high titers (1,280 to 10,240), and each antiserum showed no significant difference between the Cowden and Shintoku strains in indirect IF antibody titers (no greater than a twofold difference). These results suggest that common group antigens are highly conserved among gp C rotavirus strains (21), and all of the viruses induced antibody responses in guinea pigs. Antiserum to the Shintoku strain showed an 80-fold lower FFN antibody titer against the Cowden strain than the homologous strain. Antiserum to the Cowden strain had a 16-fold lower FFN antibody titer against the Shintoku strain than the homologous strain (Table 1). Gnotobiotic pig hyperimmune antiserum to the Cowden strain neutralized the Cowden and Shintoku gp C rotaviruses at titers of 10,240 and 320, respectively, a 32-fold titer difference (data not shown). Antisera to other gp C rotavirus strains showed two neutralization patterns. Antisera to WH, Wi, and Ehime 86-542 neutralized the Cowden strain at high titers (2,560 to 5,120) but failed to neutralize the Shintoku strain (<80). Antiserum to HF did not neutralize either the Cowden or the Shintoku strain, although this serum possessed indirect IF antibody to both strains at high titers (2,560).

Reactivities of the Cowden and Shintoku strains in both tests (IF and FFN) against convalescent-phase antisera (Table 2) were similar to those observed when hyperimmune antisera were used (Table 1). All of the antisera had indirect IF antibody to both strains at titers of 80 to 640, and each antiserum showed no significant difference between these strains in indirect IF antibody titers (no greater than a twofold titer difference). Antisera to strains Cowden and Shintoku distinguished each virus with at least 16-fold differences for the reciprocal FFN antibody titers. Antisera to other strains showed two neutralization patterns (Table 2) which were the same as those for hyperimmune antisera (Table 1). Antisera to WH, Wi, and Ehime 86 neutralized the Cowden strain at titers of 320 to 2,560 but failed to neutralize the Shintoku strain (titer, <80). Antisera to HF collected from three pigs did not neutralize either the Cowden or the Shintoku strain (titer, <80).

For gp A rotaviruses, antigenically distinct serotypes are usually determined on the basis of a 20-fold or greater difference between titers of homologous and heterologous reciprocal neutralizing antibodies (11, 38). In the present study, antisera to strain Cowden distinguished Shintoku with

TABLE 2. Indirect IF and neutralizing antibody titers of convalescent-phase sera from gnotobiotic pigs, a colostrum-deprived calf, and humans experimentally or naturally infected with group C rotaviruses against Cowden porcine and Shintoku bovine group C rotaviruses

Convalescent-phase serum		Indirect IF antibody titer[a] against rotavirus		Neutralizing antibody titer[b] against rotavirus	
Serum no.[c]	Infected species-strain	Porcine group C (Cowden)	Bovine group C (Shintoku)	Porcine group C (Cowden)	Bovine group C (Shintoku)
43-1	Porcine-Cowden	640	640	2,560	<80
B-4	Bovine-Shintoku	160	160	<80	1,280
E-421	Human-Ehime 86	640	640	320	<80
E-489	Human-Ehime 86	640	640	640	<80
E-454	Human-Ehime 86	160	160	320	<80
E-313	Human-Ehime 86	320	320	320	<80
43-11	Porcine-Wi	80	80	640	<80
15-5	Porcine-WH	160	160	1,280	<80
43-7	Porcine-WH	80	80	2,560	<80
12-10	Porcine-HF	160	80	<80	<80
15-1	Porcine-HF	160	80	<80	<80
15-2	Porcine-HF	160	160	<80	<80

[a] Determined as the reciprocal of the highest serum dilution that resulted in fluorescing cells.
[b] Determined as the reciprocal of the highest serum dilution which caused an 80% or greater reduction in fluorescent foci.
[c] Each number represents serum collected from individual animals or human patients.

16-fold (hyperimmune guinea pig antiserum) and 32-fold (convalescent gnotobiotic pig antiserum) titer differences in the neutralization tests. Similarly, antisera to strain Shintoku showed 80-fold (hyperimmune guinea pig antiserum) and 16-fold (convalescent calf antiserum) titer differences in the neutralization assays. Because the antisera to these strains distinguished each virus with at least 16-fold differences, we propose to classify the Cowden and Shintoku strains as different gp C rotavirus serotypes. This finding is the first report of the presence of serotypic variation among gp C rotaviruses based on antigenic analysis. Two neutralizing patterns against strains Cowden and Shintoku were observed when antisera to WH, Wi, Ehime 86-542, and HF were used. Our data indicate that WH, Wi, and Ehime 86-542 are more closely related antigenically than HF to the Cowden strain and all of them are antigenically distinct from the Shintoku strain. The WH, Wi, and Ehime 86-542 strains may belong to the same serotype as the Cowden strain, and additional serotypes (HF strain) may exist among gp C rotaviruses. However, final confirmation requires additional analysis by two-way cross-neutralization tests. Our preliminary findings of the serotypic similarity between Cowden and a human gp C rotavirus further supports the feasibility of using the cultivatable porcine gp C rotavirus (Cowden) and antisera to this virus as diagnostic reagents for human gp C rotaviruses to avoid the difficulty in producing reagents directly from noncultivatable human gp C rotaviruses.

Comparison of these results with those of hybridization assays using a Cowden gene 8 cDNA probe described previously (13) showed good agreement. The Cowden gene 8 probe (VP7 gene) hybridized strongly with the corresponding gene of the WH and Wi strains of porcine gp C rotavirus and a human gp C rotavirus strain isolated in Japan (88-196), but weaker hybridization signals were observed with the corresponding gene of the Shintoku and HF strains. This finding suggests that the serologic diversity observed in the present study is related to the antigenicity of VP7 of gp C rotaviruses. The data further imply that hybridization assays using VP7 gene probes for gp C rotaviruses may provide alternative methods for serotyping of noncultivatable gp C rotaviruses, similar to their use for serotyping of gp A rotaviruses (7, 15). Furthermore, genetic reassortment of noncultivatable gp C rotaviruses with strains Cowden and Shintoku may be effective for the rescue of noncultivatable gp C rotaviruses by methods similar to ones described for gp A rotaviruses (9). This procedure could provide reassortant gp C rotaviruses useful for serotypic characterization of noncultivatable strains.

We thank A. G. Agnes, K. L. Gadfield, P. A. Weilnau, and R. A. Simkins for technical assistance.

This study was supported in part by the Special Research Grants Program, Cooperative State Research Service, U.S. Department of Agriculture (competitive grant 85-CRSR-2-2590). Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

REFERENCES

1. **Arista, S., L. Giovannelli, and L. Titone.** 1985. Detection of an antigenically distinct human rotavirus in Palermo, Italy. Ann. Inst. Pasteur Virol. **136B**:229–235.
2. **Bohl, E. H., L. J. Saif, K. W. Theil, A. G. Agnes, and R. F. Cross.** 1982. Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in pigs. J. Clin. Microbiol. **15**:312–319.
3. **Bremont, M., J. Cohen, and M. A. McCrae.** 1988. Analysis of structural polypeptides of porcine group C rotavirus. J. Virol. **62**:2183–2185.
4. **Bridger, J. C., and J. K. F. Brown.** 1985. Prevalence of antibody to typical and atypical rotaviruses in pigs. Vet. Rec. **116**:50.
5. **Bridger, J. C., S. Pedley, and M. A. McCrae.** 1986. Group C rotavirus in humans. J. Clin. Microbiol. **23**:760–763.
6. **Caul, E. O., C. R. Ashleey, 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.
7. **Estes, M. K., and J. Cohen.** 1989. Rotavirus gene structure and function. Microbiol. Rev. **53**:410–449.
8. **Flewett, T. H., and G. N. Woode.** 1978. The rotaviruses. Brief review. Arch. Virol. **57**:1–23.
9. **Greenberg, H. B., A. R. Kalica, R. G. Wyatt, R. W. Jones, A. Z. Kapikian, and R. M. Chanock.** 1981. Rescue of noncultivatable human rotavirus by gene reassortment during mixed infection with *ts* mutants of cultivatable bovine rotavirus. Proc. Natl. Acad. Sci. USA **78**:420–424.
10. **Hoshino, Y., M. M. Sereno, K. Midthum, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutrali-

zation of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**:8701–8704.
11. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J. Infect. Dis. **149**:693–702.
12. **Janke, B. H., J. K. Nelson, D. A. Benfield, and E. A. Nelson.** 1990. Relative prevalence of typical and atypical strains among rotaviruses from diarrheic pigs in conventional swine herds. J. Vet. Diagn. Invest. **2**:308–311.
13. **Jiang, B. M., Y. Qian, H. Tsunemitsu, K. Y. Green, and L. J. Saif.** 1991. Analysis of the gene encoding the outer capsid glycoprotein (VP7) of group C rotaviruses by Northern and dot blot hybridization. Virology **184**:433–436.
14. **Jiang, B. M., L. J. Saif, S. Y. Kang, and J. H. Kim.** 1990. Biochemical characterization of the structural and nonstructural polypeptides of a porcine group C rotavirus. J. Virol. **64**:3171–3178.
15. **Kapikian, A. Z., and R. M. Chanock.** 1990. Rotaviruses, p. 1353–1404. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed. Raven Press, New York.
16. **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.
17. **McNulty, M. S.** 1978. Rotaviruses. J. Gen. Virol. **40**:1–18.
18. **McNulty, M. S., D. Todd, G. M. Allan, J. B. McFerran, and J. A. Greene.** 1984. Epidemiology of rotavirus infection in broiler chickens: recognition of four serogroups. Arch. Virol. **81**:113–121.
19. **Nagesha, H. S., C. P. Hum, J. C. Bridger, and I. H. Holmes.** 1988. Atypical rotaviruses in Australian pigs. Arch. Virol. **102**:91–98.
20. **Offit, P. A., and G. Blavat.** 1986. Identification of the two rotavirus genes determining neutralization specificities. J. Virol. **57**:376–378.
21. **Ojeh, C. K., B. M. Jiang, H. Tsunemitsu, S. Y. Kang, P. A. Weilnau, and L. J. Saif.** 1991. Reactivity of monoclonal antibodies to the 41-kilodalton protein of porcine group C rotavirus with homologous and heterologous rotavirus serogroups in immunofluorescence tests. J. Clin. Microbiol. **29**:2051–2055.
22. **Oseto, M., Y. Yamashita, Y. Okuyama, H. Kuwabara, and H. Inoue.** 1986. Detection of atypical rotaviruses by polyacrylamide gel electrophoresis. Igakunoayumi **136**:223–224. (In Japanese.)
23. **Pedley, S., J. C. Bridger, J. F. Brown, and M. A. McCrae.** 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol. **64**:2093–2101.
24. **Pedley, S., J. C. Bridger, D. Chasey, and M. A. McCrae.** 1986. Definition of two new groups of atypical rotavirus. J. Gen. Virol. **67**:131–137.
25. **Penaranda, M. E., W. D. Cubitt, P. Sinarachatanant, D. N. Tayler, S. Likanonsakul, L. J. Saif, and R. I. Glass.** 1989. Group C rotavirus infections in patients with diarrhea in Thailand, Nepal, and England. J. Infect. Dis. **160**:392–397.
26. **Qian, Y., B. Jiang, L. J. Saif, S. Y. Kang, Y. Ishimaru, Y. Yamashita, M. Oseto, and K. Y. Green.** 1991. Sequence conservation of gene 8 between human and porcine group C rotaviruses and its relationship to the VP7 gene of group A rotaviruses. Virology **182**:562–569.
27. **Saif, L. J.** 1990. Nongroup A rotaviruses, p. 73–96. *In* L. J. Saif and K. W. Theil (ed.), Viral diarrheas of man and animals. CRC Press, Inc., Boca Raton, Fla.
28. **Saif, L. J., E. H. Bohl, E. M. Kohler, and J. Hughes.** 1977. Immune electron microscopy of TGE virus and rotavirus (reovirus-like agent) of swine. Am. J. Vet. Res. **38**:13–20.
29. **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.
30. **Saif, L. J., L. A. Terrett, K. L. Miller, and R. F. Cross.** 1988. Serial propagation of porcine group C rotavirus (pararotavirus) in a continuous cell line and characterization of the passaged virus. J. Clin. Microbiol. **26**:1277–1282.
31. **Saif, L. J., and K. W. Theil.** 1985. Antigenically distinct rotaviruses of human and animal origin, p. 208–214. *In* S. Tzipori (ed.), Infectious diarrhea in the young. Elsevier Science Publishing, Inc., New York.
32. **Snodgrass, D. R., A. J. Herring, I. Campbell, J. M. Inglis, and F. D. Hargreaves.** 1984. Comparison of atypical rotaviruses from calves, piglets, lambs, and man. J. Gen. Virol. **65**:909–914.
33. **Terrett, L. A., and L. J. Saif.** 1987. Serial propagation of porcine group C rotavirus (pararotavirus) in primary porcine kidney cell cultures. J. Clin. Microbiol. **25**:1316–1319.
34. **Theil, K. W., C. M. McCloskey, L. J. Saif, D. R. Redman, E. H. Bohl, D. D. Hancock, E. M. Kohler, and P. D. Moorhead.** 1981. Rapid, simple method of preparing rotaviral double-stranded ribonucleic acid for analysis by polyacrylamide gel electrophoresis. J. Clin. Microbiol. **14**:273–280.
35. **Tsunemitsu, H., B. Jiang, and L. J. Saif.** 1992. Detection of group C rotavirus antigens and antibodies in animals and humans by enzyme-linked immunosorbent assays. J. Clin. Microbiol. **30**:2129–2134.
36. **Tsunemitsu, H., L. J. Saif, B. Jiang, M. Shimizu, M. Hiro, H. Yamaguchi, T. Ishiyama, and T. Hirai.** 1991. Isolation, characterization, and serial propagation of a bovine group C rotavirus in a monkey kidney cell line (MA104). J. Clin. Microbiol. **29**:2609–2613.
37. **Ushijima, H., H. Honma, A. Mukoyama, T. Shinozaki, Y. Fujita, M. Kobayashi, M. Ohseto, S. Morikawa, and T. Kitamura.** 1989. Detection of group C rotaviruses in Tokyo. J. Med. Virol. **27**:299–303.
38. **Wyatt, R. G., H. B. Greenberg, W. D. James, A. L. Pittman, A. R. Kalica, J. Flores, R. M. Chanock, and A. Z. Kapikian.** 1982. Definition of human rotavirus serotypes by plaque reduction assay. Infect. Immun. **37**:110–115.