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Use of Solid-Phase Immune Electron Microscopy for Classification of Norwalk-Like Viruses into Six Antigenic Groups from 10 Outbreaks of Gastroenteritis in the United States

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Norwalk-like viruses observed in fecal specimens from 10 outbreaks of gastroenteritis investigated in the United States between 1987 and 1992 were analyzed by solid-phase immune electron microscopy. Outbreak virus strains were classified into six antigenic groups: the four types (UK1 to UK4) previously defined in the United Kingdom, Norwalk virus, and the Oklahoma agent that was newly defined in this study. The diversity of antigenic types demonstrated in these outbreaks was greater than previously recognized and will serve as a basis for characterization of these strains at the molecular level.

Since the original report of Norwalk virus (NV) in 1972 (10), many viruses similar in shape to NV have been identified worldwide in association with gastroenteritis. These viruses usually have been named for the place where the outbreak occurred and have been tentatively grouped as the Norwalk-like viruses (NLVs). NLVs are the major cause of food-borne outbreaks of nonbacterial acute gastroenteritis in the United States (9), the United Kingdom (12, 23), and Japan (1). On the basis of reaction between acute- and convalescent-phase serum antibodies in patients and NLVs detected in their stool samples, various antigenic types have been reported in these countries by different methods and typing criteria. Three antigenic types, the NV (10, 22), the Hawaii agent (22), and the Snow Mountain agent (18), have been recognized in outbreaks in the United States. In Japan, Okada et al. reported nine antigenic types, designated small round structured virus types 1 to 9 (SRSV1 to SRSV9), determined by immune electron microscopy (IEM) (20). In the United Kingdom, Lewis reported four antigenic types (UK1 to UK4) determined by solid-phase immune electron microscopy (SPIEM) and investigated the relationship of these strains to NV, to the Hawaii, Snow Mountain, and Taunton agents and to SRSV9 from Japan (14, 15). The results of this study indicated antigenic similarities between Taunton and UK1, between NV and UK2, between Hawaii and UK3, and between Snow Mountain, UK4 and SRSV9. Lambden et al. suggested a classification of antigenic types in which type 1 is NV, type 2 is Hawaii, type 3 includes Snow Mountain and Southampton viruses, and type 4 is Taunton (12). In the United States, strains from NLV outbreaks have been classified in three groups on the basis of serological relatedness to NV observed among patients. Outbreaks have been crudely attributed to NV, to NLVs, or to unrelated agents if seroconversion determined by enzyme-linked immunosorbent assay (ELISA) was observed among more than 50%, 10 to 50%, or fewer than 10% of the patients, respectively (6). However, study of the antigenic relationships between NLVs characterized in these countries has been hampered because the viruses in this group have not yet been cultivated, no practical animal model has been developed, virus concentration in stool samples is usually very low, and specimens of human origin are nonreplenishable, variable, and in small volume.

Each year in the United States, many outbreaks of acute gastroenteritis in which fecal specimens and six or more paired serum samples are collected according to a standard protocol are investigated by state and local health departments and the Centers for Disease Control and Prevention (CDC), (3, 13). At CDC, fecal specimens are examined by electron microscopy (EM) for NLV and paired sera are tested for antibodies specific for NV (5, 17). The current study used SPIEM to examine the antigenic relatedness of NLVs identified in 10 specimens from outbreaks investigated by CDC between 1987 and 1993. To define strains and the immune response, paired sera from persons infected with the four United Kingdom reference strains and from an NV-infected volunteer were tested against the outbreak virus, and paired sera from each outbreak were tested against the four United Kingdom reference viruses and a reference NV.

Specimens were collected from cruise ships, schools, nursing homes, and restaurants investigated for epidemics of gastroenteritis. One virus stock specimen was selected from each of 10 outbreaks. The 10 virus stocks tested from the CDC collections were 1, IND/553/92/US (11); 2, IND/F6/90/US (8); 3, PIO/2442/93/US (4); 4, PEN/608/87/US (2); 5, REG/E3/89/US; 6, OKL/1217/87/US; 7, VIL/2554/93/US; 8, HEA/P1/91/US; 9, NYT/M2/91/US; and 10, WEN/K1/89/US. The code designates the CDC code/sample identification number/year/country of origin (these stocks are referred to below by CDC code/year or CDC code alone). These fecal specimens were prepared as 25% suspensions in phosphate-buffered saline (PBS; pH 7.3) by vortexing with an equal volume (1 ml) of trichlorotrifluoroethane for 30 s and centrifugation at 3,000 × *g* for 20 min. The supernatant was held at 4°C.

For reference, the four United Kingdom virus stocks were UK1a/6908/90/UK, UK2a/12121/89/UK, UK3/9802/87/UK, and UK4/5823/90/UK, where the code designates antigenic type/

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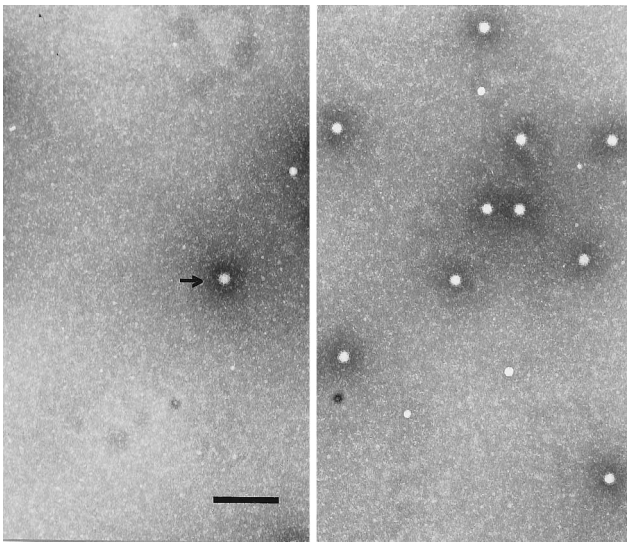

FIG. 1. An eightfold difference in virus counts between two grids, one coated with acute-phase antibody and one coated with convalescent-phase antibody. (Left) Electron micrograph of a SPIEM grid showing part of a field viewed at a microscope magnification of ×50,000. The grid was coated with protein A and acute-phase antibody from a person infected with UK2 virus and was used to capture UK2 reference virus. One virus particle is visible (arrow). Bar = 200 nm. (Right) Part of a field from a grid coated with UK2 convalescent-phase antibody and used to capture UK2 virus. Ten virus particles are visible.

sample identification number/year/country of origin. Two additional stocks of UK1 and UK2 used in some tests were UK1b/I2213/91/UK; and UK2b/1324/86/UK. Reference sera to the four United Kingdom reference strains were 1AαUK1/Taunton/79 (21), 1BαUK1/Somerset/89, 2αUK2/Somerset/89 (same patient as that with the 2a virus mentioned above), 3αUK3/Somerset/87 (same patient as that with the 3 virus mentioned above), and 4αUK4/Yorkshire/90. For NV, a reference specimen from outbreak 4 (PEN/608/87/US), previously confirmed to be NV by an ELISA and IEM (2), was used along with paired sera from an NV volunteer.

The immunoglobulin G (IgG)-based SPIEM method was used as previously described (14, 16) except that Formvar mesh carbon-coated grids (E. M. Corp., Chestnut Hill, Mass.) were precoated with protein A (Sigma, St. Louis, Mo.) by application of 20-μl drops diluted to 25 μg/ml in PBS for 30 min in a moist chamber. Purified immunoglobulin G was not prepared from the outbreak sera, but sera were used diluted to 1:1,000 in PBS. Grids were examined with either a Philips 201 electron microscope (screen magnification, ×45,000; Philips Electronic Instrument Co., Eindhoven, The Netherlands) or a JEOL 1200 EX electron microscope (screen magnification, ×50,000; Japanese Electron Optical Laboratory, Tokyo, Japan). The number of particles per unit area was counted. The unit area was standardized by counting virus particles from 5, 10, or 15 fields or the edges of one, two, or three squares. An eightfold or greater rise in virus count (Fig. 1) between grids treated with acute serum and those treated with convalescent serum was considered to indicate serological relatedness (14). All virus particles, including the newly designated Oklahoma (OKL) agent, were morphologically indistinguishable from each other and from NV (10). Viruses from each of the outbreaks were also tested for NV antigen by ELISA (7) in the laboratory of M. K. Estes, Baylor College of Medicine, Houston, Tex.

Investigation of each outbreak was done by a standard procedure. Fecal extracts were screened by direct EM to identify the specimen having the highest virus count, and paired sera from the same outbreak were then tested by SPIEM against the specimen to identify paired sera reactive with the homologous outbreak virus. The outbreak virus was then tested by paired sera specific for the four United Kingdom reference viruses, that of the NV-infected volunteer, and the OKL virus, which was found to be antigenically distinct from NV and the four United Kingdom reference viruses. Finally, the reactive paired sera from each outbreak were tested against the four United Kingdom reference viruses, the NV, and the OKL virus. This method allowed us to select sera known to react with homologous virus and to confirm results by independently testing both paired sera and virus from each outbreak with a reference panel of reagents.

We were able to type strains from the 10 outbreaks and classify these strains into six distinct antigenic groups. No one type was predominant, and the NV type accounted for only two of the 10 outbreaks investigated. The underlined boldface figures in Table 1 highlight eightfold or greater increases in counts that represent serological relatedness. Static high counts with one or more of the reference viruses were noted with some serum pairs and indicate past infection or cross-reactive antibodies. Some additional data which are not shown in Table 1 were available for several outbreaks. In the IND/92 outbreak, the virus reacted with the reference sera against NV but the paired sera from this outbreak were observed to have a rise in count both to reference NV (226-fold) and to UK3 virus (84-fold). We tested two additional serum pairs from this outbreak against NV and the UK3 virus; both gave singular rises to NV (126-fold and 46-fold) and did not react with UK3 (0/0 and 0/2). The IND/90 strain was identified to be type, UK2 and subsequent sequence analysis demonstrated the virus to be 95% homologous with UK2 in a region of the polymerase gene (8). In the PIO outbreak, one serum pair had only a 2.8-fold rise in count to the homologous virus (25 to 70), perhaps because the acute-phase sera were collected on day 7, when the titer had already begun to rise. Both pairs of outbreak sera had similar rises against UK4 virus (3.9-fold), consistent with, but not definitive for, it being antigenically related to UK4 virus. Similarly, the HEA outbreak strains were considered to be related to UK1 (an 81-fold rise with the UK1 reference sera), although definitive rises in counts with outbreak sera were not observed because of raised acute-phase counts. The PEN virus was reactive with reference sera to NV (18-fold), but not to the UK2 virus, and the antigenic relatedness to NV was confirmed by ELISA. One outbreak serum pair also had a rise in count to UK2 virus (13-fold).

The OKL and NYT outbreaks were of special interest because, despite homologous rises in counts with paired sera, neither the strains nor the paired sera could be classed as any one of the five reference types. Our finding of two outbreaks in which strains were more related to each other than to any of the reference strains established the outbreak strains to be antigenically distinct. This strain, designated OKL, was subsequently included as a new reference strain and tested against each of the other outbreak viruses.

We noted differences between the UK2 and NV viruses, which our previous work had suggested were antigenically related (14). The three outbreak viruses typed as either UK2 or NV (IND/92, IND/90, and PEN) reacted cleanly with reference sera to either UK2 or NV, but not to both. In only one instance (PEN) did outbreak sera demonstrate some cross-reactivity against both reference viruses UK2 and NV. To examine this difference further, a collection of outbreak sera with either NV or UK2 specificity were tested by SPIEM against NV viruses (PEN and IND/92) as well as UK2 viruses

TABLE 1. SPIEM results (10 outbreaks against reference virus strains)

OB[a] no.	CDC code/yr	State	Acute-phase count/convalescent-phase count for indicated combination[b]												Identity
			OB virus + reference serum of indicated antigenic type						OB serum + OB virus	OB serum + reference virus					
			UK1	UK2	UK3	UK4	NV	OKL[c]		UK1	UK2	UK3	UK4	NV[d]	OKL[e]	
1	IND/92	Hawaii	/13	/0	10/6	/7	**19/257**	3/4	**2/217**	9/18	11/4	**0/84**	7/15	**1/226**		NV
2	IND/90	Hawaii	3/12	**3/53**	1/1	/1	/1	0/0	**1/38**	31/23	**0/114**	11/15	24/28	18/24		UK2
3	PIO/93	Wyoming	8/4	/1	/0	**8/120**	/1	19/16	25/70	42/55	1/1	2/6	103/405	57/63		UK4
4	PEN/87	Pennsylvania	/1	0/4	/1	/0	**3/54**	4/13	**2/95**		**22/277**	4/6	11/11	**2/95**	0/0	NV
5	REG/89	Hawaii	/3	/3	**4/293**	4/4	/12	2/2	**4/209**			**18/161**	10/18		5/7	UK3
6	OKL/89	Oklahoma	2/1	/1	7/15	/1	8/28	**4/476**[f]	**4/476**	10/10	0/0	2/1	33/24	3/4	**4/476**[f]	OKL
7	VIL/93	Pennsylvania	**0/31**	/0	/0	0/1	1/2	/2	**3/80**	**1/188**	2/5	12/13	24/15		3/12	UK1
8	HEA/91	Washington	**3/244**	3/1	/2	/2	/2	1/2	28/107	57/212	2/0	0/1	18/30	85/61		UK1
9	NYT/91	New York	/1	/1	/2	/1	2/0	**2/197**	**5/283**			46/29		8/23	**23/294**	OKL
10	WEN/89	North Carolina	12/7	3/2	49/35	**12/190**	/1	11/23	**1/147**	10/8		2/9	**3/98**			UK4

[a] OB, outbreak.
[b] Per unit area. Acute-phase counts were not always done (space left blank) when the convalescent count was <10. Rises of more than eightfold are underlined and in boldface type.
[c] Serum from OKL OB.
[d] The PEN OB strain was used as the reference NV.
[e] The OKL OB strain.
[f] These values are repeats of those for OB serum plus OB virus, as this OB establishes a new reference strain.

(IND/90, UK2a, and UK2b) (Table 2). The NV volunteer serum reacted with both strains identified as NV and reacted in low titer with UK2b. The five paired serum samples from two NV outbreaks reacted with both NV viruses, but PEN serum 1 reacted with two of the three UK2 viruses as well. The three UK2 convalescent-phase serum samples reacted with all three UK2 strains, although one serum specimen (IND/90-3) also reacted with the PEN virus and gave a minor reaction with an additional strain (IND/92). Thus, in total, only 3 of the 40 tested cross-reactivities had an eightfold or greater rise to a strain other than the homologous virus.

The differences between NV and the UK2 strains were supported by results of the antigen ELISA for NV, as well as by antibody rises to NV. NV antigen was identified in 7 of 19 stool specimens from the IND/92 outbreak but in 0 of 8 specimens from the IND/90 outbreak. The reference strain UK2a was negative. Antibody rises to NV were identified in 10 of 12 paired serum samples from the IND/92 outbreak, 13 of 19 serum samples from the PEN outbreak, and 7 of 12 serum samples in the IND/90 outbreak. These results indicate that antibodies cross-reactive to NVs are detected more commonly by enzyme immunoassay than by SPIEM in individuals infected with the UK2 virus and that the two viruses can be distinguished with NV-specific antibodies in SPIEM or ELISA.

This survey to characterize NLVs identified in 10 outbreaks in the United States provides new information and insights about the diversity of the strains in circulation. All four antigenic types identified in the United Kingdom were present in the United States, a new strain (OKL) that was antigenically distinct from reference strains UK1-4 and NV was identified, and strains UK2 and NV were antigenically distinct. No strains that were intermediate in type (i.e., consistently reacted with more than one pair of the reference serum samples) were found, but when a range of sera were tested against viruses typed as UK2 or NV, cross-reactivity was observed with two of the nine serum samples. These heterotypic responses may be anamnestic in nature, and we speculate that they may occur more frequently between NV and UK2 because of a closer genetic and antigenic relationship between these two strains. However, it is not possible to distinguish between cross-reactivity, which would result from epitopes being shared between strains, and anamnestic responses, if only human sera are used. Our previous findings suggesting that UK2 and NV were antigenically similar (14) would seem to have been due to heterotypic responses in the reference sera, which might have been eliminated by choosing sera with a narrow type-specific response. Our results with ELISA further support this finding and indicate that the ELISA to measure seroconversion to NV was more broadly cross-reactive than SPIEM, detecting seroconversions in 7 of 12 pairs tested in the UK2 outbreaks (com-

TABLE 2. Cross-reactivities between UK2 and NV

Virus	Type	Acute-phase count/convalescent-phase count for indicated combination[a]								
		NV serum						UK2 serum		
		NV volunteer	PEN 1	PEN 2	IND/92 1	IND/92 2	IND/92 3	UK2	IND/90 2	IND/90 3
NV	PEN	**3/54**	**2/95**	ND[b]	**1/226**	ND	**0/102**	0/4	18/24	**3/105**
	IND/92	**9/87**	**3/87**	**2/124**	**2/217**	**0/126**	**0/46**	0/0	0/0	1/5
UK2	2A	0/1	**2/32**	0/0	7/6	0/0	0/1	**2/99**	**1/18**	ND
	2B	3/18	**22/277**	1/1	ND	0/0	21/40	**30/422**	**0/114**	**2/115**
	IND/90	0/0	**0/10**	0/0	ND	0/0	0/0	**1/53**	**1/38**	**0/9**

[a] Per unit area. Boldface underlined values show rises of more than eightfold.
[b] ND, not done.

pared with 23 of 31 in the NV group). ELISA for NV antigen was more specific, and none of eight specimens tested in the UK2 group were positive, compared with seven of nineteen in the NV group. These results indicate that antibodies to NV can be detected more broadly in patients infected with NLV when tested by ELISA than when tested by SPIEM, whereas the NV antigen ELISA has a narrower specificity than does SPIEM.

The NLVs studied here by SPIEM could be divided into six antigenic types. The careful use of paired sera to measure IgG rises in both reference sera against outbreak virus and in the outbreak sera against reference viruses has guarded against an arbitrary division of the strains. However, with human sera, it is not possible to control against potential heterotypic responses in sera that could further mask antigenic differences in some cases. This classification of NLVs into antigenic groups is preliminary but provides an additional biologic feature of the virus that will be useful to correlate with genogroups as more strains are sequenced (19). The value of SPIEM in the characterization of NLVs lies in its ability to detect antibodies specific for epitopes expressed on the virus surface. It has the disadvantages of being very labor intensive, because it is based on human sera which contain a wide variety of antibodies associated with past infections of the donors and depends upon nonreplenishable human reference regents. In the absence of neutralization, SPIEM may become a definitive technique for antigenic characterization when monoclonal antibodies or antibodies to recombinant proteins become more widely available.

Clearly, this study indicates that the best-characterized virus in this group, NV, represents a small proportion of a much larger group of viruses that, taken together, cause considerable morbidity in humans.

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REFERENCES

1. **Ando, T.** 1990. Small round structured viruses (SRSV): epidemiology of novel human gastroenteritis viruses in Japan. Mod. Med. Asia **36:**438–463.
2. **Cannon, R. O., J. R. Poliner, R. B. Hirschhorn, D. C. Rodeheaver, P. R. Silverman, E. A. Brown, G. H. Talbot, S. E. Stine, S. S. Monroe, D. T. Dennis, and R. I. Glass.** 1991. A multi-state outbreak of Norwalk virus gastroenteritis associated with consumption of commercial ice. J. Infect. Dis. **164:**860–863.
3. **Centers for Disease Control.** 1990. Viral agents of gastroenteritis: public health importance and outbreak management. Morbid. Mortal. Weekly Rep. **39**(RR-5)**:**1–24.
4. **Cicirello, H. G., R. Klevens, D. Lewis, C. Humphrey, R. Meuli, and R. I. Glass.** A foodborne outbreak of gastroenteritis among school children attributed to UK4 virus, a serotype of Norwalk-like virus. Am. J. Epidemiol. Submitted for publication.
5. **Gary, G. W., Jr., J. E. Kaplan, S. E. Stine, and L. J. Anderson.** 1985. Detection of Norwalk virus antibodies and antigen with a biotin-avidin immunoassay. J. Clin. Microbiol. **22:**274–278.
6. **Glass, R. I., S. S. Monroe, S. Stine, P. Madore, D. Lewis, D. Cubitt, G. Grohmann, and C. Ashley.** 1989. Small round structured viruses: the Norwalk family of agents, p. 87–90. *In* M. J. G. Farthing (ed.), Viruses and the gut. Swan Press, Ltd, London.
7. **Graham, D. Y., X. Jiang, T. Tanaka, A. R. Opekun, H. P. Madore, and M. K. Estes.** 1994. Norwalk virus infection of volunteers: new insights based on improved assays. J. Infect. Dis. **170:**34–43.
8. **Herwaldt, B. L., J. F. Lew, C. L. Moe, D. C. Lewis, C. D. Humphrey, S. S. Monroe, E. W. Pon, and R. I. Glass.** 1994. Characterization of a variant strain of Norwalk virus from a food-borne outbreak of gastroenteritis on a cruise ship in Hawaii. J. Clin. Microbiol. **32:**861–866.
9. **Kapikian, A. Z., and R. M. Chanock.** 1990. Norwalk group of viruses, p. 671–693. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman (ed.), Virology, 2nd ed., vol 1. Raven Press, Ltd., New York.
10. **Kapikian, A. Z., R. G. Wyatt, R. Dolin, T. S. Thornhill, A. R. Kalica, and R. M. Chanock.** 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. **10:**1075–1081.
11. **Khan, A. S., C. L. Moe, R. I. Glass, S. S. Monroe, M. K. Estes, L. E. Chapman, X. Jiang, C. Humphrey, E. Pon, J. K. Iskander, and L. B. Schonberger.** 1994. Norwalk virus-associated gastroenteritis traced to ice consumption aboard a cruise ship in Hawaii: comparison and application of molecular method-based assays. J. Clin. Microbiol. **32:**318–322.
12. **Lambden, P. R., E. O. Caul, C. R. Ashley, and I. N. Clarke.** 1993. Sequence and genome organization of a human small round-structured (Norwalk-like) virus. Science **259:**516–519.
13. **Lew, J. F., C. W. LeBaron, R. I. Glass, T. Torok, P. M. Griffin, J. G. Wells, D. D. Juranek, and S. P. Wahlquist.** 1990. Recommendations for collection of laboratory specimens associated with outbreaks of gastroenteritis. Morbid. Mortal. Weekly Rep. **39**(RR-14)**:**1–13.
14. **Lewis, D.** 1991. Norwalk agent and other small round structured viruses in the U.K. J. Infect. **23:**220–222.
15. **Lewis, D. C.** 1990. Three serotypes of Norwalk-like virus demonstrated by solid-phase immune electron microscopy. J. Med. Virol. **30:**78–81.
16. **Lewis, D. C., N. F. Lightfoot, and J. V. S. Pether.** 1988. Solid-phase immune electron microscopy with human immunoglobulin M for serotyping of Norwalk-like viruses. J. Clin. Microbiol. **26:**938–942.
17. **Monroe, S. S., S. E. Stine, X. Jiang, M. K. Estes, and R. I. Glass.** 1993. Detection of antibody to recombinant Norwalk virus antigen in specimens from outbreaks of gastroenteritis. J. Clin. Microbiol. **31:**2866–2872.
18. **Morens, D. M., R. M. Zweighaft, T. M. Vernon, G. W. Gary, J. J. Eslien, B. T. Wood, R. C. Holman, and R. Dolin.** 1979. A waterborne outbreak of gastroenteritis with secondary person to person spread: association with a viral agent. Lancet **i:**964–966.
19. **Norcott, J. P., J. Green, D. Lewis, M. K. Estes, K. L. Barlow, and D. W. G. Brown.** Genomic diversity of small round structured viruses in the UK. J. Med. Virol., in press.
20. **Okada, S., S. Sekine, T. Ando, Y. Hayeshi, M. Murao, K. Yabuuchi, T. Miki, and M. Ohashi.** 1990. Antigenic characterization of small, round, structured viruses by immune electron microscopy. J. Clin. Microbiol. **28:**1244–1248.
21. **Pether, J. V., and E. O. Caul.** 1983. An outbreak of food-borne gastroenteritis in two hospitals associated with a Norwalk-like virus. J. Hyg. **91:**343–350.
22. **Thornhill, T. S., R. G. Wyatt, A. R. Kalica, R. Dolin, R. M. Chanock, and A. Z. Kapikian.** 1977. Detection by immune electron microscopy of 26 to 27 nm virus-like particles associated with two family outbreaks of gastroenteritis. J. Infect. Dis. **135:**185–192.
23. **Viral Gastroenteritis Sub-Committee of the PHLS Virology Committee.** 1993. Outbreaks of gastroenteritis associated with SRSV's. PHLS Microbiol. Dig. **10:**2–8.