JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1994, p. 318–322
0095-1137/94/$04.00+0

Vol. 32, No. 2

# Norwalk Virus-Associated Gastroenteritis Traced to Ice Consumption aboard a Cruise Ship in Hawaii: Comparison and Application of Molecular Method-Based Assays

ALI S. KHAN,[1] CHRISTINE L. MOE,[1,2] ROGER I. GLASS,[1]* STEPHAN S. MONROE,[1] MARY K. ESTES,[3] LOUISA E. CHAPMAN,[1] XI JIANG,[3] CHARLES HUMPHREY,[1] EUGENE PON,[4] JOHN K. ISKANDER,[5]† AND LAWRENCE B. SCHONBERGER[1]

*Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, and Epidemiology Program Office,[5] Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; Division of Microanalytical Evaluations, Office of Plant and Dairy Foods and Beverages, U.S. Food and Drug Administration, Washington, DC 20204[2]; Baylor College of Medicine, Houston, Texas 77030[3]; and Hawaii Department of Health, Honolulu, Hawaii 96813[4]*

Received 1 June 1993/Returned for modification 31 August 1993/Accepted 26 October 1993

**Investigation of an outbreak of acute nonbacterial gastroenteritis on a cruise ship provided an opportunity to assess new molecular method-based diagnostic methods for Norwalk virus (NV) and the antibody response to NV infection. The outbreak began within 36 h of embarkation and affected 30% of 672 passengers and crew. No single meal, seating, or food item was implicated in the transmission of NV, but a passenger's risk of illness was associated with the amount of ice (but not water) consumed (chi-square for trend, $P = 0.009$). Of 19 fecal specimens examined, 7 were found to contain 27-nm NV-like particles by electron microscopy and 16 were positive by PCR with very sensitive NV-specific primers, but only 5 were positive by a new highly specific antigen enzyme immunoassay for NV. Ten of 12 serum specimen pairs demonstrated a fourfold or greater rise in antibody titer to recombinant baculovirus-expressed NV antigen. The amplified PCR band shared only 81% nucleotide sequence homology with the reference NV strain, which may explain the lack of utility of the fecal specimen enzyme immunoassay. This report, the first to document the use of these molecular method-based assays for investigation of an outbreak, demonstrates the importance of highly sensitive viral diagnostics such as PCR and serodiagnosis for the epidemiologic investigation of NV gastroenteritis.**

Norwalk virus, a member of the family *Caliciviridae*, is a common cause of outbreaks of gastroenteritis, but diagnosis of Norwalk virus infection has been difficult because of the low concentration of particles visible by electron microscopy in fecal samples and the need for reagents from infected humans to perform serologic testing (1a, 15). Recent advances in cloning and sequencing of the Norwalk virus genome have led to the development of PCR assays for the detection of Norwalk virus in fecal material and the production of recombinant baculovirus-expressed Norwalk virus (rNV) capsid antigen to assist in serodiagnosis (4, 10, 13, 14, 17). An outbreak of acute nonbacterial gastroenteritis aboard a cruise ship provided fecal and serum specimens to test these new assays as part of an investigation of an epidemic. On 22 February 1992, the Hawaii State Health Department was notified that 104 cases of acute gastroenteritis were recorded in the medical log among 694 passengers and 295 crew members aboard a 683-foot (208-m) cruise ship that toured the Hawaiian islands from 15 February to 22 February (cruise 1). The Hawaii State Department of Health requested that the ship dispose of all food, hyperchlorinate the water supply, and obtain appropriate water and fecal specimens for routine bacteriologic culture. Despite these precautions, additional cases of gastroenteritis were noted within 2 days of departure of the subsequent cruise (cruise 2; 22 February to 29 February), and an epidemiologic investigation was conducted at sea to identify the etiologic agent and determine the mode of transmission so that possible control and prevention measures could be recommended.

## MATERIALS AND METHODS

**Epidemiologic studies.** An epidemiologic investigation was conducted aboard ship and included both a review of the ship's medical log and a survey of passengers and crew members. A case of gastroenteritis was defined as any acute diarrheal illness either self-reported on questionnaires or recorded in the ship's log. Questionnaires were distributed and collected by the fifth day of the cruise from all passengers and crew members to better estimate the incidence of the disease, to determine clinical characteristics, and to identify specific exposures associated with cases that could indicate the mode of transmission.

**Sanitation inspection.** The cruise ship was inspected by sanitarians from the U.S. Food and Drug Administration who routinely examine ships involved in interstate commerce. The team inspected the sewage system; plumbing; garbage and refuse disposal systems; insect, rodent, and animal control procedures; physical facilities, including refrigeration temperatures; food protection and handling; and personal hygienic practices among the ship's crew.

**Specimens.** Fecal and acute-phase serum specimens were collected from 19 passengers and crew members who reported acute diarrhea during the last 4 days of the cruise. Convalescent-phase serum specimens were obtained from 12 of the same passengers and crew members approximately 3

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, Mailstop GO4, 1600 Clifton Road, Atlanta, GA 30333.

† Present address: Department of Pediatrics, Children's Memorial Hospital, Chicago, IL 60614.

to 4 weeks following their episodes of diarrhea. Fecal specimens were stored at 4°C, and sera were stored at −20°C until testing.

**Electron microscopy (EM).** Fecal specimens were prepared as 10% suspensions in Eagle's minimum essential medium with 0.25% paraformaldehyde. A 3-μl aliquot was applied to a 400-mesh Formvar carbon-coated grid, stained with 2% phosphotungstic acid (pH 6.5), and examined on a Phillips 201 electron microscope (18).

**RT-PCR.** Viral RNA was extracted from the fecal specimens by using the methods of Jiang et al. (12), and reverse transcription (RT)-PCR was performed on all samples with three sets of oligonucleotide primers specific for the Norwalk virus: two pairs from the polymerase region (35-36 [14] and NV51-NV3 [1]) and one pair (1-4) from the third open reading frame near the 3′ end of the genome (12, 14). Viral RNA was reverse transcribed at 42°C for 1 h, and the cDNA was amplified for 40 cycles by PCR. The amplified products were analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized with UV light. Specimens were considered positive if their PCR product comigrated with the band produced by Norwalk virus-infected fecal material from a human volunteer. Several PCR products from the polymerase region were sequenced to compare the homology of this virus with that of the reference Norwalk virus strain.

**EIA for Norwalk virus antigen.** Briefly, in the EIA for Norwalk virus antigen, 96-well microtiter plates were coated with 50 μl of rabbit hyperimmune anti-rNV serum (diluted 1:5,000), and after blocking with nonfat milk, 50 μl of the 10% stool suspensions was added. After an overnight incubation at 4°C, the plates were washed and 50 μl of a guinea pig hyperimmune anti-rNV serum specimen (diluted 1:5,000) was added. Horseradish peroxidase-conjugated goat anti-guinea pig serum and substrate were used to detect positive reactions. This antigen enzyme immunoassay (EIA) is highly specific for Norwalk virus; it does not detect other viruses in the Norwalk virus group, such as Snow Mountain agent, Hawaii agent, or the Sapporo strain of caliciviruses (11).

**Serology.** Serum specimens were tested for antibody to Norwalk virus by an EIA that uses the rNV capsid protein as antigen (7, 11, 19). Antibody units were extrapolated from a standard curve generated by using a positive control serum specimen (19). Serologic confirmation of infection was defined as a fourfold or greater increase in antibody units.

**Statistical analysis.** To examine the association of disease with specific exposures, we analyzed data from the questionnaires using Epi-Info (Version 5.01b) software to calculate relative risks (RRs) with 95% confidence intervals (CIs) (3, 25). Dose-response relationships linking disease risk with water and ice consumption were tested for significance by the chi-square test for trend or the Wilcoxon two-sample test (5, 24). Stratified Mantel-Haenszel analysis was also used to calculate RRs and 95% CIs to adjust for the effects of multiple variables (22, 23).

## RESULTS

**Epidemiologic studies.** A review of the ship's medical log indicated that the outbreak began abruptly when 93 passengers (or 13% of the total) visited the ship's physician during the last 2 days of the first cruise. On the next cruise, the outbreak continued immediately; passengers became ill within 24 h of departure, the outbreak peaked during the third day at sea, and passengers became ill throughout the cruise until arrival in port 7 days later.

TABLE 1. Attack rates for gastroenteritis among ill shipboard people as a function of daily ice ingestion, Hawaiian Islands, 22 February to 29 February 1992

| Ice consumption (no. of glasses) | No. infected/total no. (%) | |
|---|---|---|
| | Passengers[a] | Crew[b] |
| 0 | 4/31 (13) | 0/5 (0) |
| 1–2 | 53/177 (30) | 6/16 (38) |
| 3–4 | 76/226 (34) | 7/13 (54) |
| >4 | 47/121 (39) | 6/11 (55) |

[a] Chi-square for trend, $P = 0.009$.
[b] Wilcoxon two-sample test, $P = 0.04$.

A total of 608 (91%) of the 671 passengers and 64 (22%) of the 295 crew members returned questionnaires completed by the fifth day of cruise 2. Of these, 183 (30%) passengers and 19 (30%) crew members reported an acute diarrheal illness. The illness was characterized by nausea (79%), non-bloody diarrhea (76%), vomiting (70%), cramps (60%), headache (46%), muscle aches (40%), and fever (35%) and had a mean duration of 1.7 days.

The information obtained from passengers failed to implicate a single meal, food item, or specific dinner seating or dining room location as the source of the outbreak. Of the approximately 200 potential exposures evaluated, only consumption of ice was both reported by at least 50% of ill passengers and crew and was associated with becoming ill. The attack rate for passengers who consumed ice was significantly greater than that for passengers who did not (176 of 524 [34%] compared with 4 of 31 [13%]; RR = 2.60; 95% CI = 1.03, 6.55). A similar association was found for the crew (19 of 39 [49%] compared with 0 of 5 [0%]; one-tailed Fisher exact test, $P = 0.05$). In addition, this risk increased with increasing amounts of ice consumed for passengers and crew alike (Table 1). Among both passengers and crew, there was no statistically significant association for illness and consumption of water (170 of 526 [32%] compared with 6 of 32 [19%] [$P = 0.16$] and 14 of 35 [40%] compared with 5 of 13 [38%] [$P = 0.81$], respectively) or for illness and increasing amounts of water consumed. Of interest, although the water supply was hyperchlorinated and the food items present during the outbreak on cruise 1 were discarded prior to cruise 2, ice was not discarded, providing a potential common link between these outbreaks.

We also tested the hypothesis that illness was related to failure to decontaminate the cabins between cruises. During cruise 2, passengers who occupied a cabin that had been assigned to an ill passenger of the first cruise were significantly more likely to become ill than were passengers who were not residents of such cabins (48 of 113 [42%] versus 135 of 466 [29%], respectively; RR = 1.47; 95% CI = 1.13, 1.90). The association between illness and occupancy in these cabins remained after adjusting for ice consumption (RR = 1.60; 95% CI = 1.14, 2.24). These cabins were located on various decks of the ship and were not supplied by any single ice machine.

**Sanitation inspection.** The cruise ship received a score of 44 of 100 on the Food Service Establishment inspection and a summary vessel sanitation score of 70 of 100 (passing score, 85) during the sanitary inspection by investigators from the U.S. Food and Drug Administration. Water samples contained adequate levels of chlorine, and total coliforms were within acceptable levels. However, four of the five ice machines inspected, including both machines

TABLE 2. Results of diagnostic assays for Norwalk virus on 19 fecal samples from an outbreak of gastroenteritis

| No. of specimens | EM | EIA | PCR with NV51-NV3 primer pair | Serology |
|---|---|---|---|---|
| 1 | + | + | + | + |
| 1 | + | + | + | NA[a] |
| 2 | + | − | + | + |
| 3 | + | − | + | NA |
| 1 | − | + | + | + |
| 1 | − | + | − | + |
| 1 | − | + | + | NA |
| 5 | − | − | + | + |
| 1 | − | − | + | − |
| 1 | − | − | + | NA |
| 1 | − | − | − | − |
| 1 | − | − | − | NA |
| 19[b] | 7/19 | 5/19 | 16/19 | 10/12 |

[a] NA, not available.
[b] Values in this row are totals or number infected/total number of subjects.

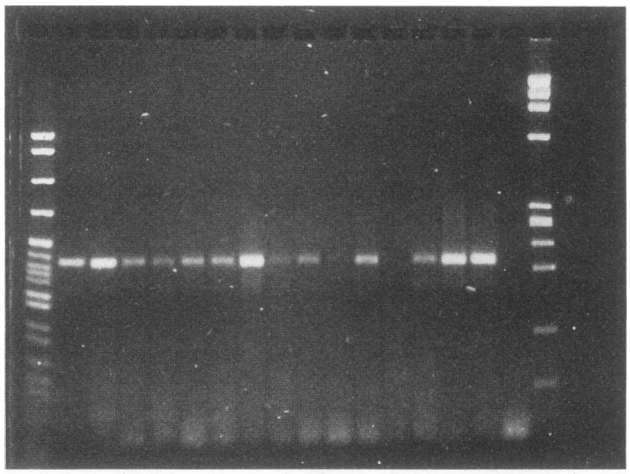


FIG. 1. UV-illuminated agarose gel showing 206-bp PCR product from 14 fecal samples (lanes 1 to 14) from ill shipboard people, a positive control (lane P; Norwalk virus-infected fecal sample from a human volunteer), and a negative control (lane N; water). The left molecular mass marker (lane M) is pBR322-Msp fragments and the right molecular mass marker (lane M) is ϕX174-*Hae*III fragments. The assay whose results are shown here was performed with 3% agarose gel with 0.5 μg of ethidium bromide per ml and was run at 140 V for 90 min.

that supplied the dining room, did not have appropriate air-gap devices to prevent sewage backup. Additionally, the ice machines were designed with a large bin covered by a hinged lid; hand-held scoops for ice removal were chained to the large bins. Inspection indicated that great care would be required to avoid touching, and hence potentially contaminating, ice when scooping it from the bins. Epidemiologically, no specific ice machine, however, was implicated as the source of the outbreak.

**Laboratory studies.** Of the 19 fecal specimens examined by direct EM, 7 contained 27-nm small round-structured viruses, indicative of a Norwalk virus-like agent. By EIA, Norwalk virus antigen was detected in 5 of 19 specimens tested, although there was concordance between the EIA and EM results for only 2 specimens (Table 2). Sixteen of the 19 fecal samples were positive by RT-PCR with one set of primer pairs (NV51-NV3; Fig. 1). All the samples were negative with the 1-4 primers, and six were weakly positive with the 35-36 primers. With the NV51-NV3 primers, PCR was more sensitive than EM or EIA. Norwalk virus RNA was found in seven samples that were negative by EM and EIA.

Because of the discordant RT-PCR results obtained with the different primer sets, purified PCR products from three samples were sequenced and compared with the sequence of the reference Norwalk virus strain to determine whether genetic variations could explain the failure of the other primers and the fecal EIA to react with these outbreak specimens. One of these samples was positive by EM, EIA, and PCR, one was positive by EM and PCR, and one was positive by PCR only. Sequence analysis of a 145-base region in the polymerase gene indicated that the three isolates from the outbreak were identical to each other and that the etiologic agent of the outbreak had only 81% nucleotide identity and an 88% amino acid identity to the reference Norwalk virus strain.

Ten of 12 paired serum specimens examined by EIA demonstrated a fourfold or greater rise in antibody titer to the rNV (Fig. 2). One serum specimen pair had a greater than threefold rise (i.e., 29 to 102) in titer, and one serum specimen pair from a crew member had a titer that was high in both acute- and convalescent-phase sera. A fecal specimen from this crew member was negative for virus by EM, EIA, and PCR. The large proportion of fourfold or greater serum antibody rises to the Norwalk virus in the outbreak (83%) was indicative of a Norwalk virus etiology.

## DISCUSSION

The large outbreak of Norwalk virus gastroenteritis described here was traced to the consumption of presumably contaminated ice. Evidence implicating ice as the most likely vehicle for the outbreak is circumstantial, but included (i) the statistically significant association of ice consumption with disease demonstrated separately for both passengers and

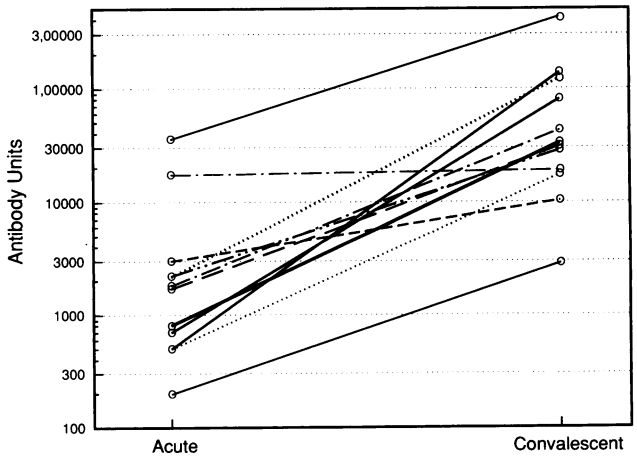


FIG. 2. Antibody response with rNV as the antigen in acute- and convalescent-phase serum specimens (19). Antibody units were extrapolated from a standard curve generated by using a positive control serum specimen and were plotted on a semilogarithmic scale.

crew, (ii) the dose-response curve for passengers and crew, (iii) the rapid onset of the outbreak after embarkation on the cruise, and (iv) the failure to clean the ice machines after the outbreak of the previous week. The lack of a statistically significant association between illness and water consumption and the failure to identify fecal contamination of the ship's water supply during initial testing suggest that the ice machines themselves, rather than the water supply, were contaminated. Inspection revealed that the ice machines could have been readily contaminated by a server's hands or possibly from sewage backup because of the lack of appropriate safety devices. Surface contamination of ice, as opposed to its water supply, has previously been identified as the source of diarrheal outbreaks caused by *Salmonella enteritidis* and *Giardia lamblia* (8, 20). Ice made from contaminated water has previously been implicated as the vehicle for transmission of cholera (6) and Norwalk virus during a multistate diarrheal outbreak (2).

Contaminated ice is unlikely to have been the sole factor in the outbreak described here. The association between illness during cruise 2 and the occupation of a cabin of persons who were ill during cruise 1 suggests the possibility of environmental spread. This association has also been observed previously in similar settings (9, 21). The combination of this finding with anecdotal reports of person-to-person spread suggests that this outbreak was multifactorial in nature. After our investigation, the cruise ship was voluntarily wet-docked for 1 week to discard ice, thoroughly clean the ice machines and cabins, and remedy all other U.S. Food and Drug Administration violations. Only four cases of gastroenteritis were reported among 873 passengers and crew members aboard the ship during the subsequent cruise, which was consistent with the usual number of such cases on a typical cruise.

Confirmation of the etiology of Norwalk virus infections in investigated outbreaks has been limited by the lack of widely available, sensitive laboratory techniques for the detection of Norwalk virus. Until recently, diagnosis relied on the observation of small round-structured virus particles in feces by EM and immune EM and demonstration of seroconversion by EIAs that used reagents from infected human volunteers. Because of the low concentration of virus in feces, direct EM is a relatively insensitive method for the detection of the majority of Norwalk virus infections (15). Even by immune EM, Norwalk virus particles were visible in only 48% of fecal samples from volunteers with diarrhea and 34% of stool specimens from serologically confirmed outbreaks of Norwalk virus gastroenteritis (15). EIAs for the detection of antibodies in serum were not widely available because of the limited supply of reagents from infected humans.

The study described here is the first to apply molecular method-based diagnostics for Norwalk virus to an investigation of an outbreak of Norwalk virus gastroenteritis. In the outbreak described here, the detection rate by direct EM was 37% (i.e., 7 of 19 specimens). PCR with one set of primer pairs (NV51-NV3) had greater sensitivity than either EM or EIA and detected seven positive specimens that were missed by the other assays. However, the successful application of RT-PCR for investigations of Norwalk virus outbreaks depends upon the choice of primers. By using primers which work for prototype Norwalk virus-infected specimens, few of the samples from the outbreak were positive with primers in the polymerase region (35-36) and none were positive with a set from the 3′ end of the virus genome (primer pair 1-4). Our experience with the NV51 and NV3 primers, which were used successfully in the analysis of the present and other outbreaks, shows that they are more sensitive than the other two primer pairs, perhaps because they share greater homology with the polymerase genes of other Norwalk-like viruses (16). The sequences of the PCR products from the polymerase region from three samples from the present outbreak indicate that there is a 19% divergence in the nucleotide sequence and a 12% divergence in the amino acid sequence from the reference Norwalk virus strain, even though in the present outbreak 83% of the 12 paired serum specimens demonstrated a fourfold or greater rise in antibody titer in serum to the baculovirus-expressed antigen.

The antigen EIA is potentially a more widely applicable diagnostic test for screening specimens from Norwalk virus outbreaks, since it requires less time, equipment, and expertise than those required by EM or PCR. However, the results of the present study indicate that the antigen EIA is not as sensitive as PCR with the appropriate primers, and the reactivity of EIA against the family of Norwalk viruses appears to be narrow (11). The results of the fecal diagnostics indicate that the antigen EIA detected fewer numbers of positive samples in comparison with EM. While two samples were positive by both EM and EIA, each assay detected Norwalk virus antigen or particles in several samples that were negative by the other assay; broken Norwalk virus particles may be detectable by EIA but may not be visible by EM. Five of the EM-positive fecal samples did not react with the antisera in the EIA. However, all of these samples were PCR positive, and paired serum samples from two of these patients were serologically positive for Norwalk virus (sera from the other patients were not available).

There was good concordance between the fecal PCR diagnostics and the results of the serologic assay. One of the two people who showed no rise in Norwalk virus antibody titer also had a fecal specimen that was negative for Norwalk virus by EM, EIA, and PCR, while the other had a threefold rise in antibody titer and a fecal specimen that was negative for Norwalk virus by EM and EIA but positive by PCR. The use of the rNV capsid protein in the EIA for serum antibodies is a valuable advance in Norwalk virus diagnostics. The assay is comparable to the previous Norwalk virus serologic assay, but it has the advantages of greater range, sensitivity, and reproducibility and does not require nonreplenishable and variable reagents from infected humans (19).

New molecular method-based assays for the detection of viral RNA by RT-PCR and Norwalk virus antibodies with expressed antigen were used to establish an etiologic diagnosis in an outbreak of acute gastroenteritis. These methods were more sensitive than previous diagnostic assays for Norwalk virus and have the potential to be broadly reactive and widely applicable for investigations of Norwalk virus-induced gastroenteritis. Sequence analyses of the Norwalk virus isolates from the present outbreak and other outbreaks will allow for the design of broadly reactive PCR primers in regions of the genome (polymerase region) known to be highly conserved among caliciviruses and will allow for the detection of other members of this group of viruses associated with outbreaks of gastroenteritis.

## ACKNOWLEDGMENTS

We thank Michelle N. Nakata, Hawaii State Health Department, and Deborah Agles for invaluable assistance during the outbreak investigation, Tamie Ando for sharing Norwalk primer pair NV51-NV3, Jon Gentsch and Baoming Jiang for advice on PCR and sequencing, Sara Stine for performing serologic studies, Robert

Holman for statistical assistance, John O'Connor for editorial assistance, and Joseph Madden for technical review.

This work was supported in part by U.S. Public Health Service cooperative agreement AI 30448 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

## REFERENCES

1. **Ando, T.** Personal communication.
1a. **Blacklow, N. R., and H. B. Greenberg.** 1991. Viral gastroenteritis. N. Engl. J. Med. **325:**252–264.
2. **Cannon, R. O., J. R. Poliner, R. B. Hirschhorn, D. C. Rodeheaver, P. R. Silverman, E. A. Brown, G. H. Talbot, S. E. Strine, S. M. Monroe, D. T. Dennis, and R. I. Glass.** 1991. A multistate outbreak of Norwalk virus gastroenteritis associated with consumption of commercial ice. J. Infect. Dis. **164:**860–863.
3. **Dean, A. G., J. A. Dean, A. H. Burton, and R. C. Dicker.** 1990. Epi-info, version 5: a word processing, database, and statistics program for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta.
4. **De Leon, R., S. M. Matsui, R. S. Baric, J. E. Herrmann, N. R. Blacklow, H. B. Greenberg, and M. D. Sobsey.** 1992. Detection of Norwalk virus in stool specimens by reverse transcriptase-polymerase chain reaction and nonradioactive oligoprobes. J. Clin. Microbiol. **30:**3151–3157.
5. **Fleiss, J. L.** 1981. Statistical methods for rates and proportions. John Wiley & Sons, Inc., New York.
6. **Glass, R. I., A. Alim, A. Eusof, J. D. Snyder, B. Jusuf, S. Anwar, Z. Bakari, C. Helmi, and B. Winardi.** 1984. Cholera in Indonesia: epidemiologic studies of transmission in Aceh Province. Am. J. Trop. Med. Hyg. **33:**933–939.
7. **Gray, J. J., X. Jiang, P. Morgan-Capner, U. Desselberger, and M. K. Estes.** 1993. Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J. Clin. Microbiol. **31:**1022–1025.
8. **Hedberg, C. W., K. E. White, J. A. Johnson, L. M. Edmonson, J. T. Soler, J. A. Korlath, L. S. Theurer, K. L. MacDonald, and M. T. Osterholm.** 1991. An outbreak of *Salmonella enteritidis* infection at a fast-food restaurant: implications of foodhandler-associated transmission. J. Infect. Dis. **164:**1135–1140.
9. **Ho, M.-S., R. I. Glass, S. S. Monroe, H. P. Madore, S. Stine, P. F. Pinsky, D. Cubit, C. Ashley, and E. O. Caul.** 1989. Viral gastroenteritis aboard a cruise ship. Lancet **ii:**961–965.
10. **Jiang, X., D. Y. Graham, K. Wang, and M. K. Estes.** 1990. Norwalk virus genome cloning and characterization. Science **250:**1580–1583.
11. **Jiang, X., T. Tanaka, A. Opekun, P. Madore, D. Y. Graham, and M. K. Estes.** Submitted for publication.
12. **Jiang, X., J. Wang, D. Y. Graham, and M. K. Estes.** 1992. Detection of Norwalk virus in stool by polymerase chain reaction. J. Clin. Microbiol. **30:**2529–2534.
13. **Jiang, X., M. Wang, D. Y. Graham, and M. K. Estes.** 1992. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J. Virol. **66:**6527–6532.
14. **Jiang, X., M. Wang, K. Wang, and M. K. Estes.** 1993. Sequence and genomic organization of Norwalk virus. Virology **195:**51–61.
15. **Kapikian, A. Z., and R. M. Chanock.** 1990. Norwalk group of viruses, p. 671–693. *In* B. N. Field and D. M. Knipe (ed.), Virology. Raven Press, New York.
16. **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.
17. **Matsui, S. M., J. P. Kim, H. B. Greenberg, S. Wanchuang, S. Qiming, P. C. Johnson, H. L. DuPont, L. S. Oshiro, and G. R. Reyes.** The isolation and characterization of a Norwalk virus-specific cDNA. J. Clin. Invest. **87:**1456–1461.
18. **Miller, S. E.** 1986. Detection and identification of viruses by electron microscopy. J. Electron Microsc. Tech. **4:**265–301.
19. **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.
20. **Quick, R., K. Paugh, D. Addiss, J. Kobayashi, and R. Baron.** 1992. Restaurant-associated outbreak of giardiasis. J. Infect. Dis. **166:**673–676.
21. **Reid, J. A., E. O. Caul, D. G. White, and S. R. Palmer.** 1988. Role of infected food handler in hotel outbreak of Norwalk-like viral gastroenteritis: implications for control. Lancet **ii:**321–323.
22. **SAS Institute.** 1990. SAS/STAT users guide, version 6, 4th ed., vol. 2. SAS Institute Inc., Cart, N.C.
23. **Schlesselman, J. J.** 1982. Case-control studies. Oxford University Press, New York.
24. **Siegel, S.** 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York.
25. **Thomas, D. G.** 1975. Exact and asymptotic methods for the combination of 2×2 tables. Comp. Biomed. Res. **8:**423–446.