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Epidemiologic, Virologic, and Host Genetic Factors of Norovirus Outbreaks in Long-term Care Facilities

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Abstract

Background—In the Unites States, long-term care facilities (LTCFs) are the most common setting for norovirus outbreaks. These outbreaks provide a unique opportunity to better characterize the viral and host characteristics of norovirus disease.

Methods—We enrolled 43 LTCFs prospectively to study the epidemiology, virology, and genetic host factors of naturally occurring norovirus outbreaks. Acute and convalescent stool, serum, and saliva samples from cases, exposed and nonexposed controls were collected. Norovirus infection was confirmed using quantitative polymerase chain reaction testing of stool samples or 4-fold increase in serum antibody titers. The presence of histo-blood group antigens (secretor, ABO, and Lewis type) was determined in saliva.

Results—Sixty-two cases, 34 exposed controls, and 18 nonexposed controls from 10 norovirus outbreaks were enrolled. Forty-six percent of acute, 27% of convalescent case, and 11% of control stool samples tested norovirus positive. Outbreak genotypes were GII.4 (Den Haag, n = 3; New Orleans, n = 4; and Sydney, n = 2) and GI.1 (n = 1). Viral load in GII.4 Sydney outbreaks was significantly higher than in outbreaks caused by other genotypes; cases and controls shed similar amounts of virus. Forty-seven percent of cases shed virus for ≥21 days. Symptomatic infections with GII.4 Den Haag and GII.4 New Orleans were detected among nonsecretor individuals.

Conclusions—Almost half of all symptomatic individuals shed virus for at least 21 days. Viral load was highest in GII.4 viruses that most recently emerged; these viruses also infect the

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Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

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nonsecretor population. These findings will help to guide development of targeted prevention and control measures in the elderly.

Keywords

norovirus; long-term care facilities; shedding; secretor status

Noroviruses are the leading cause of severe gastroenteritis world-wide [1]. An estimated 19–21 million cases of norovirus disease occur in the United States every year [2]. Noroviruses cause ≥50% of acute gastroenteritis outbreaks in long-term care facilities (LTCFs) [3, 4]. During norovirus outbreaks, all-cause hospitalizations and deaths increase by approximately 10% relative to non-outbreak periods [5]. Clinical symptoms usually start with a sudden onset of vomiting and diarrhea, lasting for up to 72 hours, with more severe outcomes observed in young children (<5 years) and older adults (≥65 years) [2].

Noroviruses (nonenveloped single-stranded, positive-sense RNA viruses) are divided into at least 6 genogroups (GI-GVI) and further subdivided into more than 38 genotypes based on phylogenetic analysis of the major capsid protein [6, 7]. GII.4 strains have been responsible for the majority of outbreaks, with a new GII.4 variant strain emerging every 2–4 years [8, 9].

Histo-blood group antigens (HBGAs) have been identified as putative attachment factors for norovirus [10]. Persons carrying a functional fucosyltransferase 2 (encoded by the *FUT2* gene) are termed secretors and express HBGAs on the gut epithelial cells, whereas homozygous individuals with 428G>A nonsense mutations at the *FUT2* gene, called nonsecretors, are almost completely protected from norovirus infections [11–16].

Data from challenge studies in healthy adults have helped to clarify how the interactions between virus, host genetics, and immunology result in norovirus illness [17–19]. Since most norovirus outbreaks occur in the elderly, we prospectively investigated outbreaks in 43 LTCFs from November 2009 to January 2013 in order to determine the epidemiology and virology of the outbreaks and the associations among norovirus disease or infection, viral genotype, and HBGAs.

MATERIALS AND METHODS

Ethics Statement

The institutional review boards of the Oregon State Public Health Division and the Centers for Disease Control and Prevention (CDC) approved the study. All residents and staff of LTCFs were eligible for inclusion, excluding those cognitively or decisionally impaired. Written informed consent was obtained from each participant.

Participant Recruitment

Prior to the onset of each winter norovirus season (December–March), LTCFs in Oregon were recruited to participate in the study. A total of 43 LTCFs were enrolled from November 2009 to January 2013. Individual study participants were recruited both before the norovirus season and when outbreaks occurred. An outbreak was suspected to be caused by norovirus

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based on clinical and epidemiologic characteristics [20]. Enrolled participants were classified as cases and exposed and nonexposed controls based on symptomatology and contact with a sick person (Figure 1). Only outbreaks that were reported to local health departments within 3 days of the onset date of the first case, had at least 10 sick individuals, had 20 potentially exposed individuals, and were still ongoing at the time of notification were selected for inclusion in the study. See Supplementary data for detailed study design and methods.

Data and Sample Collection

Clinical data were obtained directly from participants using a standardized questionnaire. The severity of gastrointestinal symptoms was assessed using a score system based on the duration and frequency of diarrhea or vomiting, fever, and treatment (Supplementary Table).

Saliva samples were collected on the day of symptom onset or exposure (day 0) from cases and exposed controls, respectively, and also on days 1, 14, and 21 to study the immune responses, which will be described in another manuscript. Serum and stool specimens were collected from cases and exposed controls on days 0 and 21 and stored at −20°C and 4°C, respectively. Single saliva, serum, and stool samples were collected from nonexposed controls within 7 days after onset of the outbreak.

Norovirus Detection, Quantification, and Genotyping

Norovirus RNA from 50 μL of 10% fecal suspension was detected using GI/GII TaqMan real-time quantitative polymerase chain reaction (RT-qPCR) [9]. The P2 domain was amplified by RT-PCR using oligonucleotide primer sets specific for GI.1 and GII.4 viruses detected in the study. Genotyping and phylogenetic analysis were performed using MEGA5 software [21] and norovirus reference sequences [6]. All stool samples were also tested for enteropathogens using xTAG Gastrointestinal Pathogen Panel (Luminex Molecular Diagnostic).

Secretor Status and HBGA Phenotyping

Secretor genotype, defined by a single nucleotide polymorphism at position 428G>A in the *FUT 2* gene, was determined by pyrosequencing as described previously [22]. HBGA phenotypes were determined from saliva by enzyme immunoassay using monoclonal antibodies (1 μg/mL) against blood group antigens A or B (Ortho Clinical Diagnostics), H antigen, Lewis (Le) a, Lewis b, Lewis x (Santa Cruz Biotechnology), or Lewis y (Calbiochem) [23]. Three saliva samples from each participant were tested for HBGA phenotypes.

To determine whether norovirus strains could bind saliva from secretor and nonsecretor individuals, 100 μL of GII.4 Den Haag, GII.4 New Orleans, or GII.4 Sydney virus-like particles (VLPs) at 0.5, 1.0, and 5.0 μg/mL were added to saliva-coated wells. VLP and saliva were genotype matched by outbreak. VLP binding was detected with polyclonal antibody against the norovirus strain and 3,3′,5,5′ tetramethylbenzidine substrate. Norovirus GII.4 Den Haag and GII.4 Sydney VLPs were kindly provided by Dr Charles

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Arntzen (Arizona State University) and Dr Ralph Baric (University of North Carolina), respectively.

Data Analysis—Nonparametric data were compared using Wilcoxon rank sum, and categorical variables were analyzed using Fisher exact test. Log transformation and 1-way analysis of variance followed by Tukey multiple comparison test was applied to viral loads to compare mean values. Spearman correlation was used to compare illness and shedding duration with severity score. Shedding duration was analyzed as Kaplan–Meier survival probability. GraphPad Prism 5 (Graph PadSoftware Inc., California) was used to perform analyses, and *P* values <.05 were considered statistically significant.

RESULTS

Demographics

From November 2009 through January 2013, 39 norovirus outbreaks were reported at 30 (70%) of 43 LTCFs (Supplementary Figure). Ten (26%) of 39 outbreaks met the inclusion criteria of our study and resulted in 386 illnesses, 29 hospitalizations, and 5 associated deaths.

Sixty-two cases (65% aged ≥70 years), 34 exposed controls (9% aged ≥70 years), and 18 nonexposed controls (5% aged ≥70 years) were enrolled (Table 1). Most cases were LTCF residents (79%), whereas the exposed control group predominantly consisted of staff who provided direct patient care (68%), and 50% of the nonexposed controls were nonpatient care staff. Symptoms included diarrhea (84%), fatigue (81%), vomiting (76%), and nausea (74%). Presence of both vomiting and diarrhea (62%) was reported more frequently than either symptom alone (14% and 22%, respectively) (Table 2). Adults aged ≥70 years were more likely than younger patients to report both symptoms (73% vs 45%; $P = .035$). Two (3%) of 62 cases reported to have had a similar illness within the preceding year.

Illness duration was longer in cases aged ≥70 years (n = 29; median, 4; interquartile range [IQR], 3–4) than aged <70 years ($P = .041$), with 19 (60%) lasting >3 days and 4 (13%) lasting >5 days (Figure 2*A*). The median severity score was 5.0 (n = 62; range, 2–10). The median score was higher in those aged 50–69 and 70–89 years (median, 6) than in those aged 21–49 and ≥90 years (median, 5; Figure 2*B*). No correlation was observed among severity score, illness, or shedding duration for the group or after age stratification (n = 29; Figure 2*C*).

Norovirus Detection and Genotyping

The 10 studied outbreaks occurred in 7 LTCFs, with more than 1 outbreak detected in 2 LTCFs (Table 3). Norovirus was detected in stool samples from 42 cases, 4 exposed controls, and 1 nonexposed control. No sequence variation among samples from the same outbreak was detected except for outbreaks A, F, and H. Phylogenetic analysis of P2 sequences (Figure 3) showed that 18 (95%) of the 19 cases in outbreak A were typed as GII. 4 New Orleans and 1 case as GII.4 Den Haag. Positive samples from exposed and nonexposed controls (A_268_Exp_Cv and A_205_NonExc, respectively) as well as convalescent stool samples had 100% sequence homology with the outbreak strain.

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One (H_552) of 3 samples from outbreak H (GII.4 New Orleans) had 1 amino acid substitution in the P2 sequence. Sequences from the same participant at days 0 and 21 were identical.

Three of the 4 samples from outbreak F genotyped as GI.1, while the 4th sample (F_446_Ac) was typed as GII.4 New Orleans. However, since the convalescent stool sample from F_446 was also typed as GI.1, the GII.4 New Orleans virus at day 1 was considered unrelated to the outbreak.

Virus Shedding and Coinfections

Among 179 stool samples, 104 (55 acute, 49 convalescent, 47 acute/convalescent pairs) were collected from 62 cases (Table 3). Forty-two (76%) of 55 acute and 20 (42%) of 49 convalescent samples tested positive for norovirus. Six acute GII.4 New Orleans–positive stool samples also tested positive for *Clostridium difficile* toxin A/B (n = 5) and *Giardia* (n = 1).

A total of 59 stool samples (29 acute, 30 convalescent, 26 acute/convalescent pairs) were collected from 34 exposed controls. Of these, 3 (10%) of 29 acute and 1 (3%) of 30 convalescent tested positive for norovirus. One (6%) of 16 stool samples from nonexposed controls tested positive for norovirus.

The median collection date after onset of symptoms for acute positive stool was 3 days (n = 42; range, 0–8), with no significant difference among genotypes ($P = .732$). No significant differences were found between the mean acute viral loads in samples from cases in outbreaks with the same genotype (Table 3). In contrast, compared with outbreaks caused by GII.4 New Orleans ($P = .029$), GII.4 Den Haag ($P = .009$), and GI.1 Norwalk ($P = .0281$), the mean viral load in GII.4 Sydney outbreaks (2.33×10^{13} copies/g stool; range, 0.17–4.49×10^{13} copies/g stool) was significantly higher (Figure 4). Similarly, the mean viral load in GII.4 New Orleans outbreaks (7.81×10^{10} copies/g stool; range, 4.33–18.4×10^{10} copies/g stool) was significantly higher than in GII.4 Den Haag ($P = .008$) and GI.1 Norwalk ($P = .0065$) outbreaks. No difference was found between mean viral load from GI.1 Norwalk and GII.4 Den Haag outbreaks ($P = .4695$). Mean acute viral shedding was comparable in cases (n = 40; 1.3×10^{12} copies/g stool) and asymptomatic exposed controls (n = 3; 1.22×10^{12} copies/g stool; $P = .4611$). No correlation was observed between viral load and age ($r = 0.1891$; $P = .2031$).

Prolonged shedding (≥21 days) was detected in 16 (47%) of the 35 cases with positive acute stool. The mean acute viral load (3.02×10^{12} copies/g stool) among cases with prolonged shedding was not significantly different from those that had a negative convalescent stool (5.91×10^{10} copies/g stool; $P = .1012$; Figure 5*A*). Nevertheless, cases with acute titers $\geq 10^{10}$ copies/g stool (n = 13) were significantly more likely to remain positive compared with those shedding $<10^{10}$ copies/g stool ($P < .001$), with 9 (70%) lasting for at least 4 weeks (Figure 5*B*).

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HBGAs and Disease

Of 114 individuals, 103 (90.4%) were secretors, of which 42 (40.4%) were homozygous and 61 (59.6%) were heterozygous. To investigate potential associations between HBGAs and norovirus infection or disease, the ABO, Lewis, and secretor status distributions among cases and exposed controls (n = 96) were stratified by infection (infected or noninfected) and disease (symptomatic or nonsymptomatic) and compared for each genotype or variant. Eighty-nine (93%) of 96 participants were secretor (Le^{a-b+}). All ABO types were detected among cases and exposed controls. Among 7 nonsecretors, 5 were Le^{a+b-} (Lewis positive) and 2 were Le^{a-b-} (Lewis negative). The 18 nonexposed controls included both secretors (n = 14; ALe^{a-b+} n = 7; ALe^{a-b-} n = 1 and OLe^{a-b+}, n = 6) and nonsecretors (n = 4, Le^{a+b-}).

The blood type A/secretors (n = 4) of the GI.1 outbreak were symptomatic. Fifty-five (94.8%) of 58 symptomatic individuals in GII.4 outbreaks were secretors. However, secretors were as likely as nonsecretors to be infected. In GII.4 Den Haag outbreaks, 15 (60%) of 25 secretors were infected compared with 1 (50%) of 2 nonsecretors ($P = 1.0$). Similarly, 39 (80%) of 49 secretors and 2 (66%) of 3 nonsecretors were infected with GII.4 New Orleans viruses ($P = .520$), whereas GII.4 Sydney infected 5 (55%) of 9 secretors, but none of 2 nonsecretors ($P = .455$). In GII.4 outbreaks, we found that individuals regardless of blood type were infected, regardless of the GII.4 variant. In summary, no association between secretor status or blood type and symptomatic infection was found for GII.4 viruses.

Three of 7 nonsecretors were infected by either GII.4 Den Haag (n = 1; Le^{a+b-}) or GII.4 New Orleans (n = 2; Le^{a+b-} and Le^{a-b-}). To determine if nonsecretor individuals could be infected, saliva samples from all participants were incubated with different GII.4 VLPs (Figure 6). All VLPs bound to saliva samples from secretors. GII.4 Den Haag VLPs also bound to saliva samples from nonsecretors (n = 3, median, 0.432, range, 0.250–0.533; Figure 6*A*). In contrast, GII.4 New Orleans and Sydney VLPs were able to bind saliva from nonsecretors Le^{a+b-}, but not from Le^{a-b-} (Lewis negative), when the VLP concentration was increased to 5.0 μg/mL. Five of 6 Le^{a+b-} saliva samples were from controls, suggesting that lack of infection in these individuals could be the result of exposure to small amounts of virus. VLPs did not bind to saliva from either an exposed control (I_561) or a case (A_309) who were nonsecretor Le negative (Le^{a-b-}).

DISCUSSION

In the United States, the number of individuals aged >65 years is expected to grow to 79.7 million (21% of the population) by 2040 [25], of which a substantial number will live in LTCFs. Since more than 60% of reported norovirus outbreaks occur in the elderly population [4], targeted vaccination of high-risk groups such as the elderly may significantly reduce the burden of hospitalization and death. During our 3-year study, 70% of the prospectively enrolled LTCFs reported at least 1 norovirus outbreak. No associations between severity of disease, illness duration, and virus shedding was found. Viral loads were as high as $>10^{13}$ copies/g stool and were significantly higher in outbreaks caused by GII.4 New Orleans and GII.4 Sydney viruses shortly after these variants had emerged [9, 26]. Symptomatic and asymptomatic individuals shed similar amounts of virus regardless of

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duration of shedding. Almost 50% of the cases shed virus for at least 3 weeks in high titers, suggesting that some individuals are able to clear norovirus infection more rapidly than others. Our data showed that nonsecretor individuals could also be infected by GII.4 Den Haag and GII.4 New Orleans viruses. This observation was further supported by confirmation that saliva from nonsecretor Le^{a+b-} individuals did bind to GII.4 Den Haag and GII.4 New Orleans VLPs.

An interesting observation in our study was the fact that although individual virus loads were similar or higher (range, 10^5–10^{13} genomic copies/g of stool) than noted in previous reports [18, 19, 27–30], the highest viral loads correlated with the recent emergence of new GII.4 variants. Viral load in acute asymptomatic individuals (exposed controls) was also relatively higher for GII.4 Sydney. Because outbreaks with higher viral load occurred relatively soon after the emergence of GII.4 New Orleans in 2009 and GII.4 Sydney in 2012, increased viral load in both symptomatic and asymptomatic individuals may suggest more efficient virus replication.

After recovery from clinical symptoms, infected individuals can still shed virus for weeks [18, 19, 30, 31]. In our study, 47% of cases who provided convalescent stool were positive at days 21–32. The mean viral load in convalescent samples (10^8 copies/g stool) was much higher than reported in other studies (10^4/g), suggesting that shedding could extend for several more weeks in these populations [32]. Although the viral load in convalescent samples was significantly higher than the minimum infectious dose [33, 34], reports from nosocomial norovirus outbreaks showed that primarily symptomatic patients contributed to transmission of disease [28, 29].

A key finding of our study was that more recent GII.4 variants can infect nonsecretors as well as secretors. Nonsecretor individuals have near-complete resistance to GI.1 and GII.4 infection; however, with lower risk, they can also be infected by GI.3 and GII.2 strains [11, 13, 15, 17]. We found that saliva from Le^{a+b-} nonsecretors bind GII.4 Den Haag, GII.4 New Orleans, and GII.4 Sydney VLPs, supporting the concept that more recent GII.4 variants are able to bind glycans from Lewis-positive nonsecretor individuals [35]. This may explain the increased number of outbreaks when GII.4 Den Haag emerged in 2006. In contrast, no increase in outbreak activity was observed when GII.4 New Orleans and GII.4 Sydney emerged, which may be a consequence of similar affinity for HBGAs [36, 37].

We also found a Lewis-negative (Le^{a-b-}) individual who was symptomatically infected, in contrast to other reports [12, 14]. These differences could be linked to different genotypes detected in each study or the presence of an HBGA-like substance on the surface of the gut that provides noroviruses with the opportunity to infect intestinal cells [38, 39].

Our study had several limitations. First, lack of norovirus detection in stool samples from several cases may have been due to late collection, intermittent shedding, or shedding below the detection level. Our ability to detect significant associations between secretor status and infection/disease was limited by small numbers of non-secretors and by small numbers of participants affected by GII.4 Sydney outbreaks. Individual behavior and different exposure scenarios may have influenced participant classification as cases or controls, since

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healthcare workers were probably consciously more careful than residents when in contact with a sick person.

In summary, we prospectively studied 10 norovirus outbreaks in LTCFs and confirmed that infected people can asymptomatically shed virus at high levels for at least 3 weeks. Although secretor status is considered an important factor of innate protective immunity, the most recent GII.4 strains appear to be able to infect the nonsecretor population as well. Future studies are needed to better understand whether protection or susceptibility is related to immune response [40]. Whether the shedding of viruses after 3 weeks represents infectious virus is another key question that will need to be answered. Such studies will help us to better understand the dynamics of norovirus outbreaks in the elderly and guide development of targeted prevention and control measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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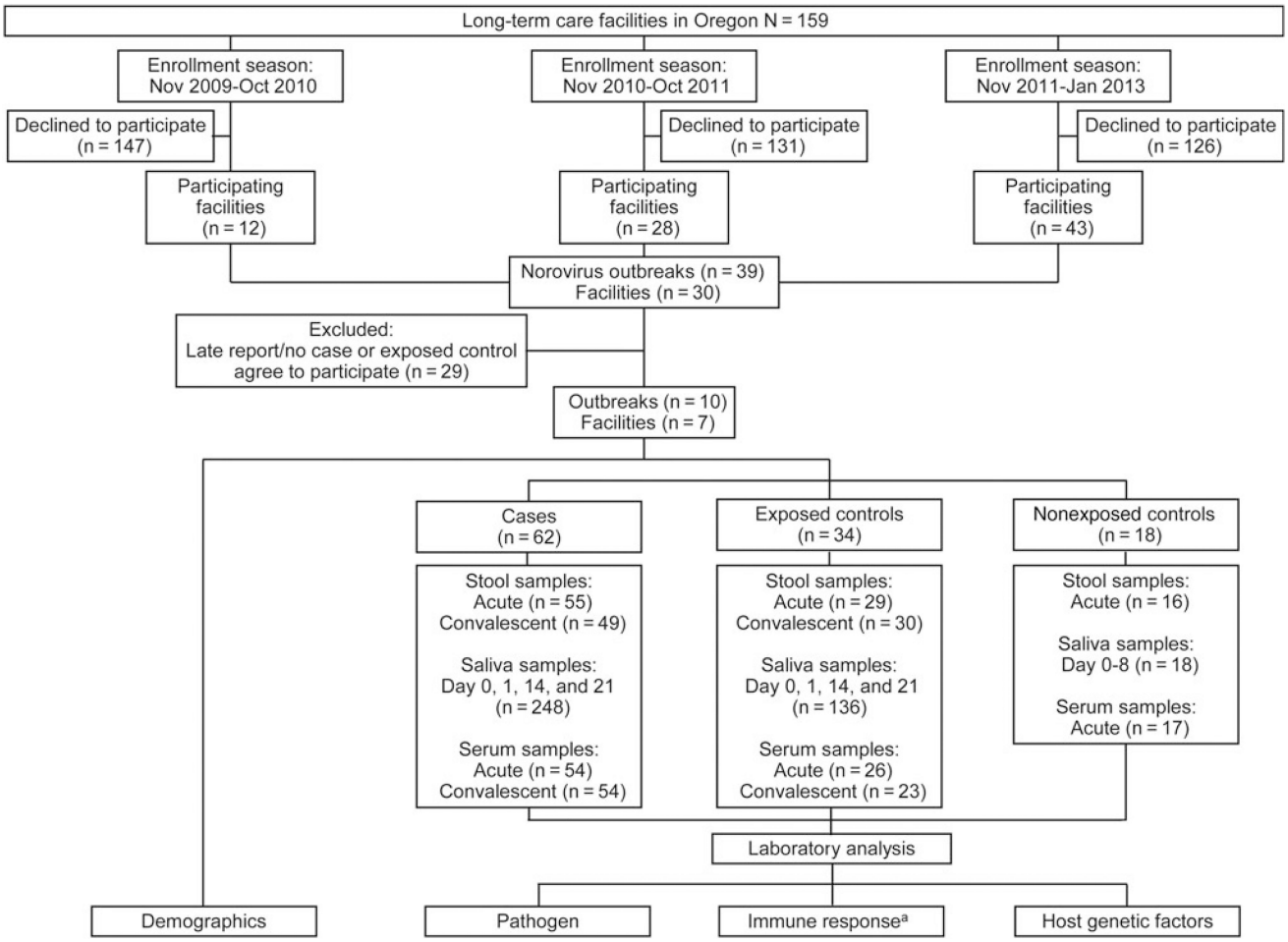

Figure 1.

Flow diagram of the study experimental design. From 2009 through 2013, 43 long-term care facilities were enrolled, and 10 norovirus outbreaks that qualified for inclusion in the study were reported. A total of 62 cases, 34 exposed controls, and 18 nonexposed controls were enrolled based on the presence of clinical symptoms and exposure to a case. [a]The immune responses will be described in another article.

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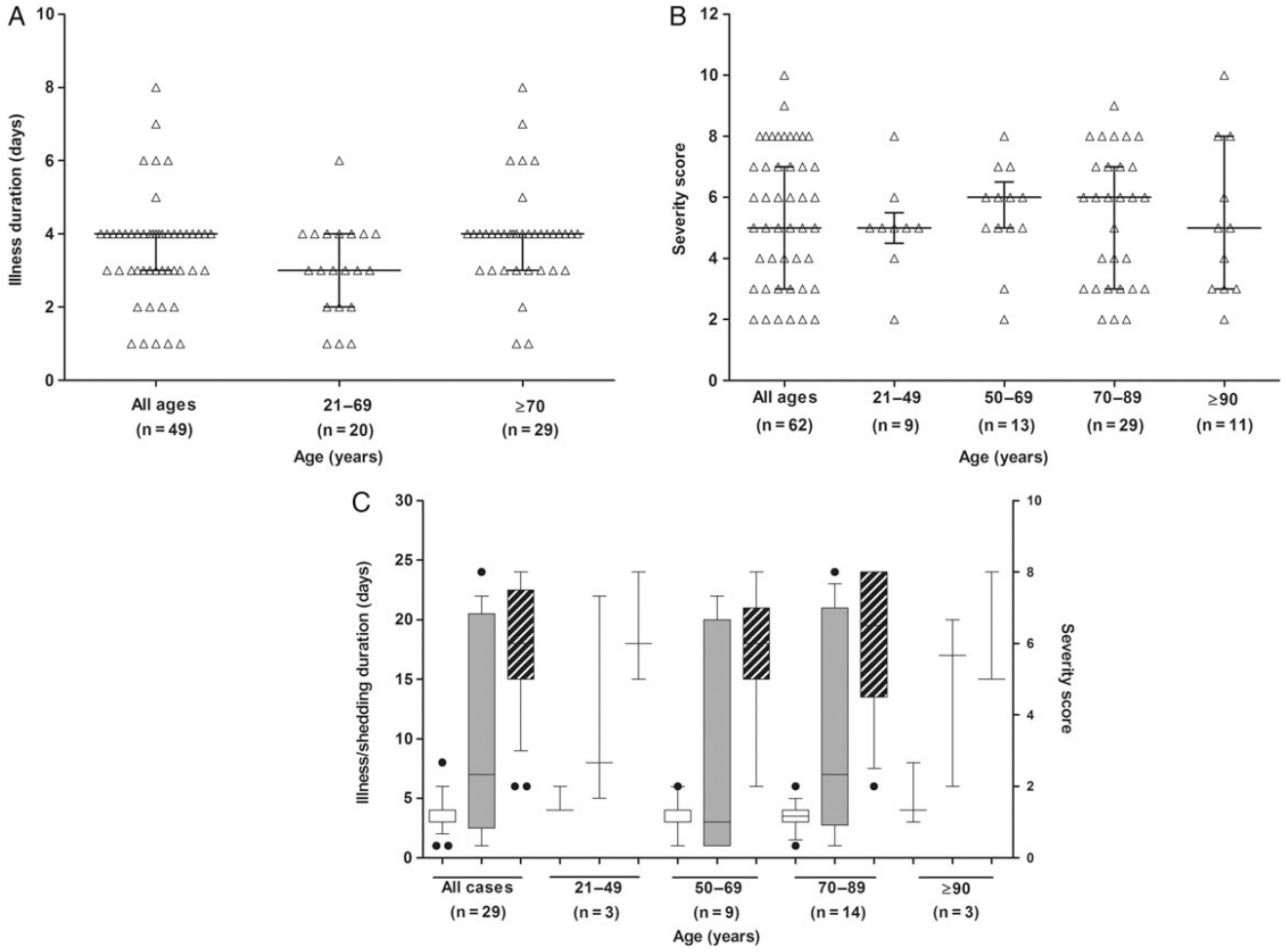

Figure 2.

Duration of illness, virus shedding, and severity score in cases reported during the study. *A*, Illness duration data from 49 cases were based on questionnaire responses. Graph represents median + interquartile range (IQR) for each age group. *B*, The severity of norovirus disease was assessed for all cases (n = 62) on the basis of a score system; scores ranged from 0 to 17, with higher scores indicating more severe disease (Supplementary Table 1). Graph represents median + IQR for each age group. *C*, Illness duration, virus shedding duration, and severity score were obtained from 29 cases from which both acute and convalescent stool samples were collected. Boxes represent 25th percentile, median, and 75th percentile, and the whiskers show the 10th–90th percentile for illness duration (white boxes), virus shedding duration (gray boxes), and severity score (stripe boxes).

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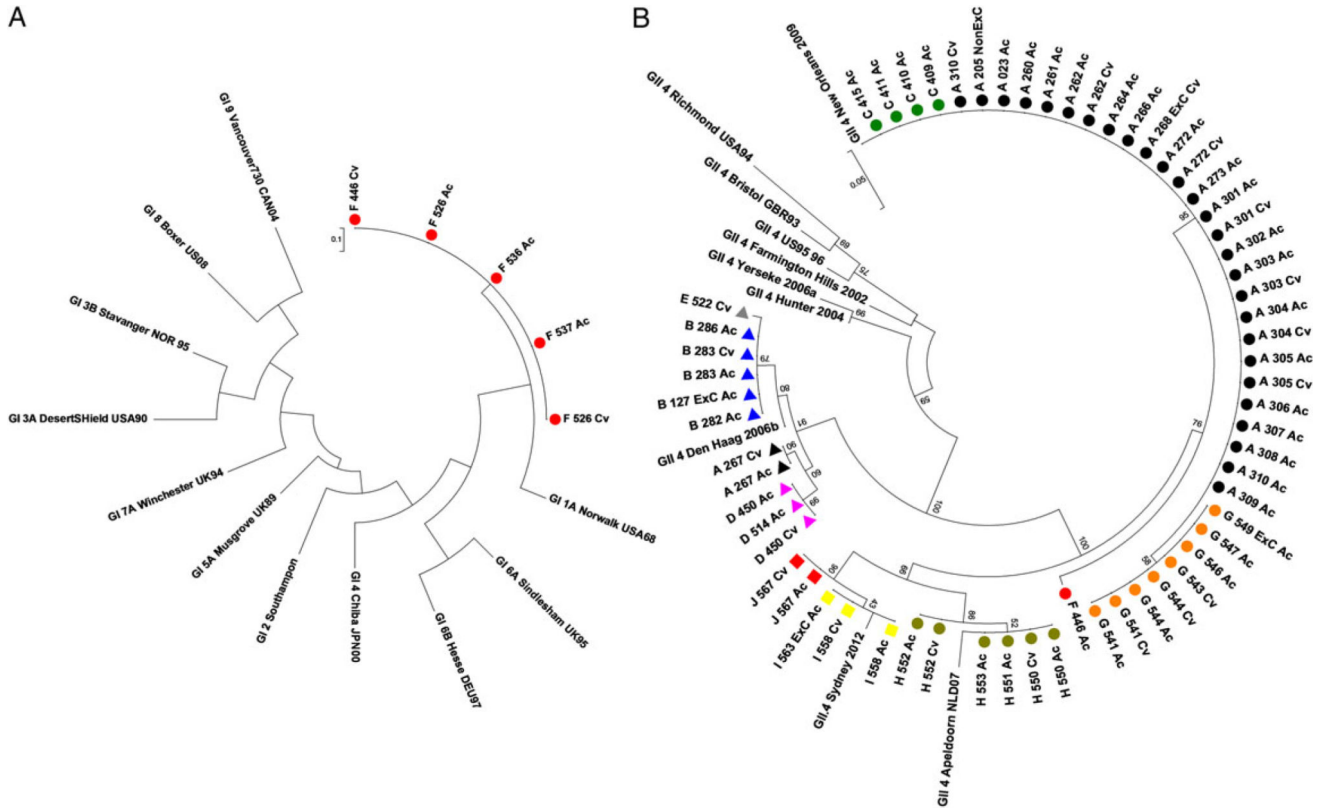

Figure 3.

Phylogenetic analysis of (*A*) norovirus GI strains (outbreak F) and (*B*) norovirus GII strains (outbreaks A–E and G–J) based on amino acid sequences of the P2 domain of major capsid protein. Samples are listed by outbreak ID, followed by participant ID and collection time (Ac, acute; Cv, convalescent). Reference sequences are labeled by their name. The evolutionary history was inferred by using the maximum likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JDT model and then selecting the topology with superior log likelihood value [24]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA5 [21]. All sequences from cases, except where indicated. GenBank accession numbers of reference sequences are as follows: GI.1 Norwalk (M87661), GI.2 Southampton (L07418), GI.3 Desert Shield (U04469), GI.3b Stavanger (AF145709), GI.4 Chiba (AB042808), GI.5 Musgrove (AJ277614), GI.6 Hesse (AJ277615), GI.7 Winchester (AJ277609), GI.8 Boxer (AF538679), GI.9 Vancouver 730 (HQ637267), GII.4 Bristol (X76716), GII.4 Richmond (GU4453253), GII.4 US95/96 (AJ0048642), GII.4 Farmington Hills 2002 US (AY4856423), GII.4 Hunter 2004 AUS (AY8830962), GII.4 Yerseke 2006a NLD (EF1269632), GII.4 Den Haag 2006b NLD (EF1269652), GII.4 Apeldoorn_2007 (AB445395), GII.4 New Orleans 2009 (GU445325), and GII.4 Sydney 2012 (JX459908). Abbreviations: ExC, exposed control; non-ExC, nonexposed control.

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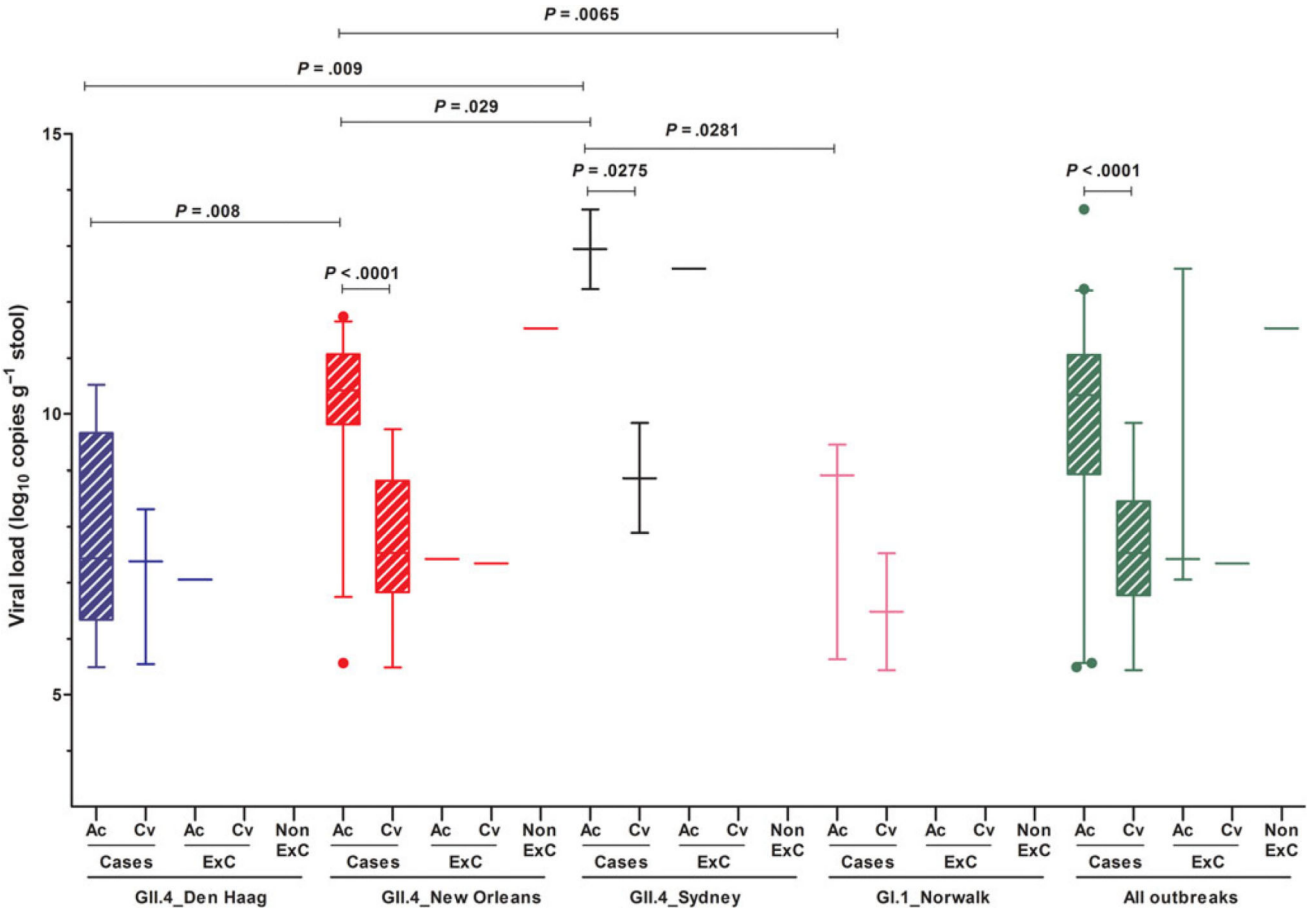

Figure 4.

Viral shedding during norovirus outbreaks reported during the study. Box plots represent $\log_{10}$ viral shedding (median and 25th and 75th quartiles [box], 10th and 90th percentiles [whiskers]). Data were stratified for cases/exposed controls/nonexposed controls, during acute (day 0–8) or convalescent (day 17–32) time with the same genotype. Only positive samples are included; therefore, lack of value indicates negative samples or sample not collected (Table 3). Classification was based on virus genotype detected in stool sample. Viral shedding was $\log_{10}$ transform and analysis of variance 1-way followed by Tukey multiple comparison test was applied to compare all mean values ($P < .05$). Abbreviations: Ac, acute; Cv, convalescent; ExC, exposed control; non-exC, nonexposed control.

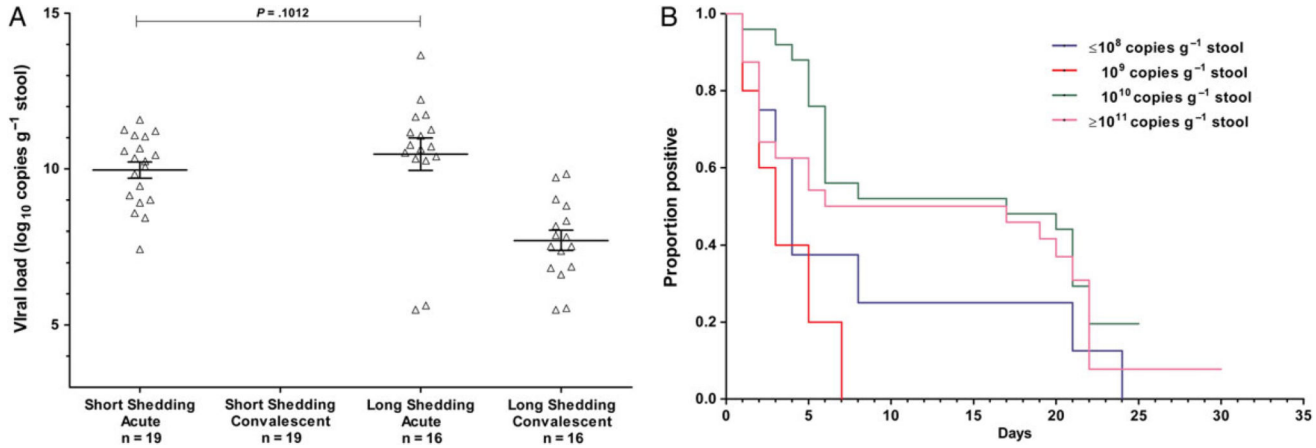

Figure 5.

Shedding duration among cases. Acute (day 0–8) and convalescent stools sample (day 17–32) were collected from 35 cases. *A*, Norovirus was detected in 16/35 convalescent samples. Data represent mean viral load (copies/g stool) plus standard deviation. Short shedding convalescent samples were negative for norovirus. *B*, Proportion of positive samples stratified by norovirus concentration in acute stool samples. The survival distributions were significantly different (log rank test, $\chi^2 = 12.45$, $P < .05$). Cases with an initial shedding higher than 10^{10} copies per gram stool were significantly more likely to continue shedding virus for 3–4 weeks ($P < .001$).

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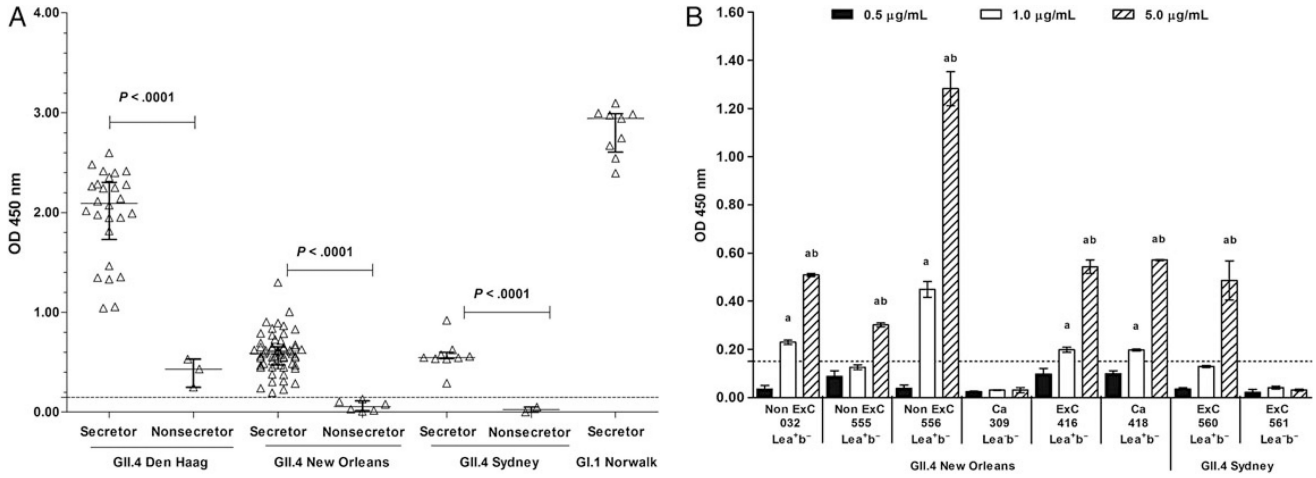

Figure 6.

Binding of norovirus virus-like particle (VLP) to saliva samples collected during the study. Saliva samples were assayed for their ability to bind outbreak-specific norovirus VLPs. *A*, Norovirus VLP (0.5 μg/mL) binding to saliva samples collected from secretors (n = 103) and nonsecretors (n = 11) during norovirus outbreaks reported during the study. Triangles represent corrected optical density (OD) (450 nm) from individual saliva samples stratified for secretor status and outbreak-specific norovirus genotype. All cases/exposed controls/nonexposed controls were included. Line represents median + interquartile range. Saliva from secretors and nonsecretors were able to bind GII.4 Den Haag, but not GII.4 New Orleans or GII.4 Sydney VLPs. *B*, Saliva samples from nonsecretors (negative on A) were assayed for their ability to bind increasing amounts of genotype-specific norovirus VLPs (0.5, 1.0, 5.0 μg/mL). Significant differences were observed after incubation of 1.0 μg/mL or 5.0 μg/mL with saliva samples from Le^{a+b-} (Lewis positive) ($P < .0001$), but not from Le^{a-b-} (Lewis negative). Abbreviations: Ca, case; ExC, exposed control; non-ExC, nonexposed control.

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Table 1

Demographic Characteristics of Study Participants

	Cases (N = 62)		Exposed Controls (N = 34)		Nonexposed Controls (N =18)	
Characteristic	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Sex						
Male	15	24	5	15	0	0
Female	47	76	29	85	18	100
Race						
Black	1	2	2	6	0	0
White	59	95	26	76	16	89
Asian	0	0	4	12	1	6
Hispanic	1	2	1	3	1	6
Native American	0	0	1	3	0	0
Unknown	1	2	0	0	0	0
Age, y						
21–49	9	14	23	67	10	56
50–69	13	21	8	24	7	39
70–89	29	47	3	9	1	5
>90	11	18	0	0	0	0
Median [min, max]	81	[25–95]	44	[22–85]	49	[21–88]
Group class						
Resident	49	79	2	6	1	6

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Characteristic	Cases (N = 62)		Exposed Controls (N = 34)		Nonexposed Controls (N = 18)	
	No.	(%)	No.	(%)	No.	(%)
Patient care staff	12	19	23	68	8	44
Nonpatient care staff	1	2	6	18	9	50
Household members	0	0	3	9	0	0

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Table 2

Frequency of Symptoms, Medical Care, and Treatment Among Cases (N = 62)

	All Ages (N = 62)		21–49 y (N = 9)		50–69 y (N = 13)		70–89 y (N = 29)		≥90 y (N = 11)	
Variable	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Symptoms										
Vomiting	48	76.2	5	55.6	7	53.8	27	90.0	9	81.8
Duration (days)										
1–4	48	100.0	5	100.0	7	100.0	27	100.0	9	100.0
>6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Episode/day										
1	14	29.2	3	60.0	0	0.0	8	29.6	3	33.3
2–4	23	47.9	1	20.0	4	57.1	12	44.4	6	66.7
>5	11	22.9	1	20.0	3	42.9	7	25.9	0	0.0
Diarrhea	53	84.1	8	88.9	12	92.3	24	80.0	9	81.8
Duration (days)										
1–4	51	96.2	8	100.0	10	83.3	24	100.0	9	100.0
5	1	1.9	0	0.0	1	8.3	0	0.0	0	0.0
>6	1	1.9	0	0.0	1	8.3	0	0.0	0	0.0
Episode/day										
1–3	22	41.5	4	50.0	9	75.0	6	25.0	3	33.3
4–5	14	26.4	1	12.5	3	25.0	8	33.3	2	22.2
>6	17	32.1	3	37.5	0	0.0	10	41.7	4	44.4

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	All Ages (N = 62)		21–49 y (N = 9)		50–69 y (N = 13)		70–89 y (N = 29)		≥90 y (N = 11)	
Variable	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Vomiting and diarrhea	39	61.9	4	44.4	6	46.2	22	73.3	7	63.6
Blood in stools	1	1.6	0	0.0	0	0.0	1	3.3	0	0.0
Fever	19	30.2	2	22.2	4	30.8	9	30.0	4	36.4
Shaking chills	25	39.7	3	33.3	6	46.2	12	40.0	4	36.4
Myalgia	27	42.9	7	77.8	9	69.2	10	33.3	1	9.1
Headache	31	49.2	4	44.4	12	92.3	10	33.3	4	36.4
Abdominal pain	41	65.1	9	100.0	9	69.2	18	60.0	5	45.5
Nausea	47	74.6	6	66.7	11	84.6	22	73.3	7	63.6
Fatigue	51	81.0	6	66.7	12	92.3	22	73.3	10	90.9
Illness duration (days)[a]										
Mean [95% confidence interval]	3.5	[3.13–3.97]	2.8	[1.88–3.61]	3.3	[2.35–4.15]	3.8	[3.04–4.58]	4.1	[3.43–4.82]
Median [interquartile range; range]	4	[3–4;1–8]	3	[2–3.75;1–8]	3.5	[2.25–4;1–6]	4	[3–4;1–8]	4	[4;3–6]
Medical care										
None	59	95.2	9	100.0	13	100.0	27	93.2	10	90.9
Emergency department	1	1.6	0	0.0	0	0.0	1	3.4	0	0.0
Hospitalization	2	3.2	0	0.0	0	0.0	1	3.4	1	9.1
Treatment										
Oral rehydration	13	20.6	3	33.3	2	15.4	6	20.0	2	18.2
Intravenous fluids	2	3.2	0	0.0	0	0.0	1	3.3	1	9.1
Perceived illness severity										

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	All Ages (N = 62)		21–49 y (N = 9)		50–69 y (N = 13)		70–89 y (N = 29)		≥90 y (N = 11)	
Variable	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Mild	13	20.6	4	44.4	3	23.1	5	16.7	1	9.1
Moderate	29	46.0	5	55.6	8	61.5	13	43.3	3	27.3
Severe	20	31.7	0	0.0	2	15.4	11	36.7	7	63.6
Last similar illness[b]										
<2 wk	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
4–12 mo	2	3.2	1	11.1	0	0.0	1	3.3	0	0.0
>1 y	31	49.2	5	55.6	7	53.8	15	50.0	4	36.4
Never	4	6.3	0	0.0	3	23.1	0	0.0	1	9.1
Unknown	25	39.7	3	33.3	3	23.1	13	43.3	6	54.5

[a] Information available from 49/62 cases (age 21–49 years [n = 8], 50–69 years [n = 12], 70–89 years [n = 21], and ≥90 years [n = 8]).

[b] Last time as reported by the participant.

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Table 3

Summary of Diagnostics and Genotyping of 10 Norovirus Outbreaks in Long-term Care Facilities

Outbreak	Long-term Care Facility	Date	Group	No. Participant Enrolled	Stool Samples No. Positive/No. Collected (%)		Viral Load[a] (RNA Copies/g Stool)		Genotype[b]
					Acute	Convalescent	Acute	Convalescent	
A	v	March 2010	Cases	24	19[c,d]/22 (86)	8[c]/19[e] (42)	4.33×10^{10}	1.76×10^{8}	GII.4 New
			Exposed controls	5	0/3 (0)	1/4 (25)	-	2.20×10^{7}	Orleans
			Nonexposed controls	11	1/10 (10)	0/0 (0)	3.40×10^{11}	-	
B	viii	May 2010	Cases	6	3/4 (75)	1/3 (33)	2.20×10^{8}	3.50×10^{5}	GII.4 Den Haag
			Exposed controls	4	1/3 (33)	0/2 (0)	1.13×10^{7}	-	
			Nonexposed controls	0	0/0 (0)	0/0 (0)	-	-	
C	xviii	January 2011	Cases	6	4[f]/6[g] (67)	0/4 (0)	1.10×10^{11}	-	GII.4 New
			Exposed controls	5	0/5 (0)	0/5 (0)	-	-	Orleans
			Nonexposed controls	0	0/0 (0)	0/0 (0)	-	-	
D	xi	April 2011	Cases	5	2/4 (50)	1/3 (33)	1.65×10^{10}	2.40×10^{7}	GII.4 Den Haag
			Exposed controls	7	0/6 (0)	0/6 (0)	-	-	
			Nonexposed controls	2	0/2 (0)	0/0 (0)	-	-	
E	v	September 2011	Cases	2	0/2 (0)	1/2 (50)	-	2.04×10^{8}	GII.4 Den Haag
			Exposed controls	1	0/1 (0)	0/1 (0)	-	-	
			Nonexposed controls	0	0/0 (0)	0/0 (0)	-	-	
F	xv	November 2011	Cases	5	4/5[c] (80)	2/5 (40)	1.21×10^{9}	1.69×10^{7}	GI.1 Norwalk
			Exposed controls	2	0/2 (0)	0/2 (0)	-	-	
			Nonexposed controls	2	0/1 (0)	0/0 (0)	-	-	
G	v	April 2012	Cases	6	4[d]/4 (100)	3/5 (60)	9.43×10^{10}	3.67×10^{8}	GII.4 New
			Exposed controls	2	1/1 (100)	0/2 (0)	2.60×10^{7}	-	Orleans
			Nonexposed controls	1	0/1 (0)	0/0 (0)	-	-	

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Outbreak	Long-term Care Facility	Date	Group	No. Participant Enrolled	Stool Samples No. Positive/No. Collected (%)		Viral Load[a] (RNA Copies/g Stool)		Genotype[b]
					Acute	Convalescent	Acute	Convalescent	
H	xix	April 2012	Cases	4	4[d]/4 (100)	2/4[e] (50)	1.84×10^{11}	2.65×10^{9}	GII.4 New
			Exposed controls	1	0/1 (0)	0/1 (0)	-	-	Orleans
			Nonexposed controls	2	0/2 (0)	0/0 (0)	-	-	
I	xvi	November 2012	Cases	3	1/3 (33)	1/3 (33)	4.49×10^{13}	3.43×10^{9}	GII.4 Sydney
			Exposed controls	4	1/4 (25)	0/4 (0)	3.90×10^{12}	-	
			Nonexposed controls	0	0/0 (0)	0/0 (0)	-	-	
J	xv	January 2013	Cases	1	1/1 (100)	1/1 (100)	1.70×10^{12}	7.70×10^{7}	GII.4 Sydney
			Exposed controls	3	0/3 (0)	0/3 (0)	-	-	
			Nonexposed controls	0	0/0 (0)	0/0 (0)	-	-	
Total				114	46/100 (46)	21/79 (27)			

[a] Mean.

[b] Outbreak genotype was established based on P2 region from positive stool samples collected from cases.

[c] At least 1 sample has a different genotype from that of the outbreak strain.

[d] Coinfection with *Clostridium difficile* toxin A/B was detected in 3, 1, and 1 acute samples (outbreak A, G, and H, respectively).

[e] *Clostridium difficile* toxin A/B was detected in 1 convalescent norovirus-negative stool sample.

[f] Coinfection with *Giardia* was detected in 1 sample.

[g] *Clostridium difficile* toxin A/B was detected in 1 acute norovirus-negative stool sample.