Supplementary data

**MATERIALS AND METHODS**

**Subject recruitment**

Prior to the onset of each norovirus season, nursing homes and assisted living facilities (together called long-term care facilities [LTCFs]) in Oregon were recruited to participate in the study. A total of 43 LTCFs in the state of Oregon were enrolled from November 2009 to January 2013. Individual study subjects were recruited by study staff, both before the norovirus season began (baseline) and when norovirus outbreaks occurred. Participating LTCFs reported suspected norovirus outbreaks to the local health department, per standard public health reporting requirements. An outbreak was suspected to be caused by norovirus based on clinical (e.g., vomiting in > 50% of cases, fever to vomit ratio < 1, or diarrhea to vomit ratio < 2.5) and epidemiologic characteristics [[1](#_ENREF_1)]. Only outbreaks that were reported to the local health departments within 3 days of the onset date of the first case, have at least 10 sick individuals and 20 potentially exposed individuals, and were still ongoing at the time of notification were selected for inclusion in the study.

Potential study subjects were screened for eligibility and asked for informed consent. Those enrolled were classified as cases or controls based on symptoms **(Fig 1)**. A case was defined as a resident or staff member with ≥3 episodes of diarrhea or vomiting in 24 h. Controls were assigned to one of two groups. An “exposed control” was a staff member or resident who remained asymptomatic despite regular contact or having shared living space with a case for at least 3 days during illness. Asymptomatic staff members and residents without such exposure to a case were recruited as “non-exposed controls”; this group was generally restricted to non-patient care staff, such as administrative personnel. Controls were not specifically matched to cases, although roughly equal numbers of cases and exposed controls were sought for enrollment in each outbreak.

**Clinical data and sample collection**

Clinical data were obtained directly from participants interviewed using a standardized questionnaire. Data were collected on date of onset (case) or date of contact with case (exposed controls), clinical symptoms and their duration, care and treatment received, and demographic information. The severity of gastrointestinal symptoms was assessed using a modified Vesikari score with a maximum of 17-points based on the duration of diarrhea or vomiting, the maximum number of diarrhea and vomiting episodes within 24 h, fever and the treatment received (Supplementary Table 1).

Saliva samples were collected on the day of symptom onset (day 0) or exposure (day 0) from cases and exposed controls, respectively, and also on days 1, 14 and 21 to study the salivary immune responses which will be described in another manuscript. Saliva was collected by rubbing the gums with an Oracol sampler sponge (Malvern Medical Developments, Worcester, UK) for 1 min, and stored at -80°C. Acute and convalescent sera were collected from cases and exposed controls on days 0 and 21 and stored at -20°C. Stool specimens were collected from cases and exposed controls on days 0 and 21 and kept at 4°C. If a specimen could not be obtained on a given day, a sample was collected as close as possible to the scheduled day. For non-exposed controls,one saliva sample, one serum sample and one stool sample were collected within 7 days after the first case identified in the outbreak.

**Norovirus detection, quantification and genotyping**

RNA was extracted from 50 µL of clarified 10% fecal suspension using the KingFisher® instrument and MagMAX™ - 96 Viral RNA Isolation Kit (Ambion, Austin, TX), according to the manufacturer’s instructions. Norovirus RNA was detected by GI/GII TaqMan real-time RT-PCR (RT-qPCR) [[2](#_ENREF_2)], and positive samples were genotyped using conventional RT-PCR followed by sequencing of the RT-PCR products [[3](#_ENREF_3)]. Standard curves were generated using 10-fold serial dilutions of GI.4 and GII.4 T7 RNA transcripts. The number of RNA copies in a fecal sample was determined based on the Ct value and corresponding number of RNA copies/µL extrapolated from the appropriate standard curve (GI or GII).

##### P2 domain amplification: The P2 domain was amplified by using the Qiagen One-Step RT-PCR kit™ (Qiagen, Valencia, CA). Oligonucleotide primer sets P2GI-1F/P2GI-1R [[4](#_ENREF_4)] and EVP2F/ EVP2R [[2](#_ENREF_2)] were used to amplify GI.1 and GII.4 viruses detected in the study, respectively. RT-PCR conditions included reverse transcription at 50°C for 30 min and denaturation at 95°C for 2 min, followed by 40 cycles of PCR at 94°C for 30 s, 55°C for GII.4 (or 47°C for GI.1) for 30 s, 68°C for 2 min, and a final extension step of 68°C for 10 min. Amplified RT-PCR products were visualized on ethidium bromide-stained 2% agarose gels.

**Bi-directional Sequencing and Genotyping:** Region C and P2 domain RT-PCR products were gel purified (QIAquick PCR purification Kit, Qiagen, Valencia, CA), sequenced using the BigDye Terminator Kit 1.1 (PE Applied Biosystems) and cleaned using the BigDye Xterminator (Applied Biosystem). Sequences were edited by Sequencher® (Gene Core Corporation, Ann Arbor, MI) and genotyped against norovirus reference sequence databases at CDC [[5](#_ENREF_5)] using MEGA [[6](#_ENREF_6)].

**Other enteric pathogens:** All stool samples were also tested for the following gastroenteric pathogens: Adenovirus 40/41, Rotavirus A, *Clostridium difficile* toxin A/B, *Campylobacter* spp., *Escherichia coli* O157, enterotoxigenic *E. coli* heat-labile enterotoxin/heat-stable enterotoxin, *Salmonella* spp., Shiga-toxin producing *E. coli*, Shiga-like toxin (Stx)1/2, *Shigella* spp., *Vibrio cholerae*, *Cryptosporidium* spp., and *Giardia* spp. using the xTAG® Gastrointestinal Pathogen Panel (Luminex Molecular Diagnostic, Toronto, Canada) according to the manufacturer’s instructions. Data acquisition and analysis were performed on MAGPIX® instruments using TDAS.

**Secretor status and HBGA phenotyping**

**Saliva processing**: Oracol tubes were thawed on ice and centrifuged at 360 x g for 5 min at 10°C and 1000 x g for an additional 5 min. Supernatant (saliva) was carefully removed, transferred to a microcentrifuge tube and stored at -80 °C. The cell pellet of buccal cells was resuspended in PBS and stored at -20°C.

**Secretor status:** Secretor genotype, defined by a single nucleotide polymorphism (SNP) at position 428 (G→A) in the *FUT 2* gene, was determined by pyrosequencing as previously described [[7](#_ENREF_7)]. Briefly, DNA was extracted from 200 µL of buccal cells using QIAampDNA Blood Mini kit (Qiagen, Hilden, Germany) and PCR was performed with FUT2\_428BIO (5’-BIOTIN-GATGGAGGAGGAATACCGCCAC-3’) and FUT2\_428R (5’-TGGGCCTCCTCCCGCACGT-3’). The pyrosequencing reaction was carried out with FUT2\_428seq (5’-GGT GGT GGT AGA AGG TC-3’) and the dispensing order GCTCAGAGC in a PyroMark Q24 instrument (Qiagen, Valencia, CA). Samples were classified as homozygous secretor (SeSe), heterozygous secretor (Sese), or non-secretor (sese).

**HBGA phenotyping:** HBGAs phenotypes were determined from saliva by EIA using monoclonal antibodies [[8](#_ENREF_8)]. Saliva samples were tested in triplicate, or three samples from the same participant were tested when available. Twenty microliters of saliva was boiled at 95°C for 10 min and centrifuged for 10 min at 13000 x g. Supernatants were diluted 1:100 in PBS and coated into EIA plates for 4 hr at room temperature. After washing, 200 µL blocking buffer (5% Non-Fat Dry Milk/PBS/0.05%Tween) was added, and plates were incubated overnight at 4°C. Monoclonal antibodies (MAb) to blood group antigens A or B (Ortho Clinical Diagnostics, Rochester, NY) were diluted 1:20, and MAb to H, Lewis a, Lewis b, Lewis x (Santa Cruz Biotechnology, CA), or Lewis y (Calbiochem, Gibbstown, NJ) were used at 1 µg/mL. PBS was used as negative control. Anti-HBGA monoclonal antibodies were incubated for 1 h, followed by a cocktail of anti-mouse IgG /IgM-AP conjugate (Sigma Chemicals) for 30 min and color development with SIGMAFAST™ p-Nitrophenyl phosphate (Sigma Chemicals). Between each incubation, plates were washed 3 times with PBS and 0.05%Tween-20), all dilutions were prepared in blocking buffer and all incubations were conducted at room temperature. A reaction was considered positive if the mean corrected optical density (ODMAb – ODPBS) was greater than three times the mean of the ODPBS well.

**VLP-Saliva binding assay:** To determine whether outbreak strains could bind saliva from secretor and non-secretor individuals, boiled saliva samples were coated into 96-well plates. After blocking, 100 µL of GII.4 Den Haag, GII.4 New Orleans or GII.4 Sydney-VLPs at 0.5, 1.0 and 5.0 µg/mL were added to saliva-coated wells. VLP and saliva were matched by outbreak. VLP binding was detected with polyclonal antibody against the norovirus strain and TMB substrate. Norovirus GII.4 Den Haag and GII.4 Sydney-VLPs were kindly provided by Dr. Ralph Baric (University of North Carolina) and Dr. Charles Arntzen (Arizona State University).

**Data analysis**: Non parametric data were compared with Wilcoxon rank-sum and categorical variables were analyzed with Fisher's exact test. Log transformation was applied to viral loads and one-way ANOVA followed by Tukey’s multiple comparison test 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 was used to perform analyses, and P-values <0.05 were considered statistically significant [[9](#_ENREF_9)] .

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