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A norovirus outbreak in a nursing home: Norovirus shedding time associated with age

Chao-Chih Lai[a], Ying-Hsueh Wang[a], Ching-Yi Wu[b], Ching-Hsiang Hung[b], Donald Dah-Shyong Jiang[b], Fang-Tzy Wu[b,c,*]

[a] Taipei City Hospital, Ren-Ai Branch, Taiwan
[b] Centers for Disease Control, Department of Health, Taiwan
[c] Department of Biotechnology and Laboratory Science in Medicine, National Yang-Ming University, Taipei, Taiwan

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ABSTRACT

Background: Norovirus (NoV) GII.4 has been identified as predominant in outbreaks in the long-term health-care facilities.
Objectives: NoV excretion during an outbreak of gastroenteritis affecting 19/42 residents and 12/33 employees was investigated in a Taiwan nursing home.
Study design: Real-time reverse transcription polymerase chain reaction (RT-PCR) was used to quantify viral RNA from stool samples up to the point of negative detection.
Results: Initial fecal viral loads in affected residents were higher than in affected employees ($p = 0.024$). Viral reduced rate was measured as 0.66/day, with a viral half-life of 1.7 days. A mixed model indicated that time (days post-illness onset), initial virus load and resident status (as opposed to employee status) were the most important determining factors of fecal NoV concentration. According to a univariable accelerated failure time (AFT) model, strong associations existed between virus excretion duration and both age ($p = 0.005$) and resident status ($p = 0.004$). No associations were noted between viral excretion duration and either initial viral load or diarrhea duration. According to a multivariable AFT model, age was the only factor affecting virus excretion duration.
Conclusion: In conclusion, outbreaks in nursing homes may have resulted from environmental contamination, the existence of asymptomatic residents and prolonged virus shedding time in the elderly and care providers. This outbreak finished quickly because frequent cleaning of the surface was done and contact precautions were taken for prolonged viral shedding residents.

1. Background

Noroviruses (NoVs) cause the majority of non-bacterial gastroenteritis outbreaks worldwide.[1] Of the five NoV genogroups, three are pathogenic to humans: GI, GII and GIV.[2] Recently the GII.4 strain has been identified as predominant in outbreaks in a large number of long-term health-care facilities.[3] Researchers have reported prolonged NoV excretion durations in immunosuppressed patients and children,[4–6] but only a limited number of studies on excretion periods and reduced rates have been performed. According to three research teams, between 28% and 80% of all gastroenteritis outbreaks occur in nursing homes and elder care facilities.[7–9] Here, we report our findings for a February 2009 outbreak in a Taipei nursing home.

2. Objectives

Our goals were to determine excretion duration and magnitude for virus titer shedding and reduced rates in infected patients.

3. Study design

3.1. Case definition

Suspected cases were defined as any resident or employee having one or more episodes of diarrhea and/or vomiting in the presence or absence of other symptoms between 18 February and 5 March 2009. Cases were confirmed by laboratory tests as described below.

3.2. Epidemiological investigation

The nursing home building has two wings with connecting hallways. It includes three single rooms, three twin rooms and 10

* Corresponding author at: Centers for Disease Control, Department of Health, 161 Kunyang Street, Nan Gang District, Taipei 115, Taiwan. Tel.: +886 2 26531362.
E-mail address: fang@cdc.gov.tw (F.-T. Wu).

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four-bed rooms. All 42 residents including those who were mobile ate their meals in their own rooms. A total of 33 employees ate their meals in the station of nursing homes. Our cohort study included nursing home residents (all suffering from dementia or stroke) and employees. The mean resident age was 73.8 (±19.2) years. Only 5 of 42 were mobile (using wheelchairs); all others were bedridden. Resident data were obtained from medical records and nurse employee interviews. Self-administered questionnaires were distributed to all employees to screen for gastroenteritis symptoms. Only confirmed cases were enrolled in the study. The study was approved by the Institutional Review Board.

3.3. Specimen collection and laboratory tests

Stool specimens were collected from 31 suspected cases and examined for *Shigella*, *Campylobacter*, *Salmonella*, *Vibrio* spp. and viruses (rotavirus, sapovirus and astrovirus).[10] No positive test results were obtained. The same specimens were used for NoV-specific reverse transcription polymerase chain reaction (RT-PCR) assays using the G2SKF/G2SKR primer set as previously described.[11,12] We also collected stool specimens from asymptomatic residents and employees for NoV testing. Regardless of the presence or absence of symptoms, specimens were collected once every 3–7 days from 42 residents and 33 employees whose stools tested positive for NoV. The first specimen was collected after symptom onset and was defined as the initial viral load. Specimen collection lasted from illness onset until negative RT-PCR results were obtained. In addition, 50 environmental specimens were collected either at the surface of the telephone, door handle, table, curtain, water bottle in the room and nursing station, or toilet, tap and trash can in the restroom by viral swabs on 24 February for NoV RT-PCR testing.

The G2SKF/G2SKR primer pair amplifiers a 344-bp amplicon, which covers the N-terminal domain and a part of the shell domain of VP1. RT-PCR products were purified using QIAquick gel extraction kits (Qiagen, Hilden, Germany), and nucleotide sequences were determined with an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA). Multiple nucleotide sequences were aligned and analyzed phylogenetically using MEGA 4.0 software.

3.4. Viral load measurements

Stool specimens were prepared with phosphate-buffered saline in 10% (w/v) suspensions clarified by centrifugation (10,000 rpm for 30 min). Environmental swab specimens were suspended in buffer according to the manufacturer's instructions (Copan, Brescia, Italy). Viral RNA was extracted from 200 μl of both kinds of clarified suspension by using MagNA Pure Compact Nucleic Acid Isolation Kits (Roche, Mannheim, Germany) according to the manufacturer's instructions. Real-time RT-PCR was performed using the COG2F/COG2R primer set as previously described.[13] The real-time RT-PCR amplicon was cloned into a pGEM-T Easy Vector (Promega, Madison, WI, USA) as a standard control. The complementary DNA (cDNA) viral load was quantified in triplicate/run by 10-fold serial dilutions (10^8–10^1 copies) plasmid standard. The NoV GII detection limit of the assay was 10^1 cDNA copies/rx equivalent to 3.2×10^4 copies/g of stool. Viral loads below the quantification limit were calculated as one-half of that limit.

3.5. Control policy

Infection control strategies were instituted, which included the reinforcement of hand hygiene, implementation of contact precautions for all case-residents, the use of masks and isolation gown and exclusion of symptomatic staffs from work until 48 h after the resolution of their symptoms. During the outbreak, the environmental surfaces of the facility were cleaned using diluted hypochlorite (bleach) every day.[3,14] All visitors were asked to wash their hands and to wear masks and isolation gowns provided by us while entering the facility during the period of outbreak, but no exclusion of visitors was done.

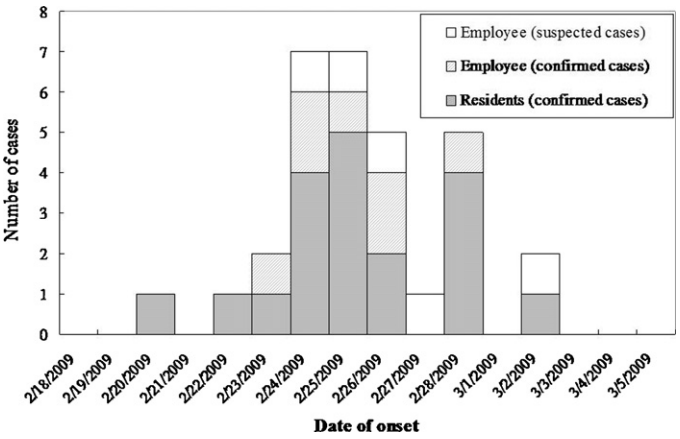

Fig. 1. Epidemic curve for the gastroenteritis ($n = 31$) outbreak in terms of onset date. A suspected case was defined as any resident or employee having one or more episodes of diarrhea and/or vomiting in the presence or absence of other symptoms. A confirmed case was defined as any diarrhea and/or vomiting case with laboratory confirmation of norovirus in stool by RT-PCR.

3.6. Statistical analysis

Data were entered into Epi Info software v3.43 (US Centers for Disease Control and Prevention, Atlanta, GA, USA) and analyzed with SAS v9.1. Categorical variables are reported as proportions. Between-group p values were calculated using Chi-squared or Fisher's exact tests. Medians were compared using Wilcoxon rank-sum tests, and means were compared using Student's t-tests. All statistical tests were two-sided; p values less than 0.05 were considered statistically significant.

Log_{10} viral load transformations were performed to reduce data skewness. Mixed models were used to determine relationships between fecal virus concentrations and illness duration, age, gender, initial viral load and resident/employee status with Kenward–Roger's method for tests of the fixed effects. Individuals are referred to as random effects in estimating fixed effects. It was also used to estimate the duration of virus shedding and viral load at onset date. NoV excretion duration data were analyzed as Kaplan–Meier survival probability estimates. Covariate effects on virus shedding time (i.e., time to stool conversion) were evaluated using an accelerated failure time (AFT) model with a Weibull distribution.[15] Univariable analysis results with p values less than 0.20 were used for backward procedure multivariate AFT analyses. Parameter coefficients connected with the AFT model are reported as percentage difference in virus shedding time, using equation $[e^{\beta} - 1] \times 100\%$.

4. Results

Over a period of 11 days, 19 of the 42 residents (45.2%) and 12 of the 33 employees (36.4%) showed symptoms of acute gastroenteritis (Fig. 1). Among those resident cases, three out of five mobile residents were affected. For these cases, their symptoms included diarrhea (100%), abdominal pain (54.5%), general weakness (45.5%), nausea (45.5%), loss of appetite (45.5%), vomiting (36.4%), headache (18.2%) and fever (9.1%). Symptoms of case-residents reported by nurses included diarrhea (80.0%) and vomiting (79.0%). The mean duration of illness was 2.0 (±1.0) days for case-residents and 1.8