Clinical Infectious Diseases

MAJOR ARTICLE

Sources of Airborne Norovirus in Hospital Outbreaks

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Background. Noroviruses are the major cause of viral gastroenteritis. Disease transmission is difficult to prevent and outbreaks in health-care facilities commonly occur. Contact with infected persons and contaminated environments are believed to be the main routes of transmission. However, noroviruses have recently been found in aerosols and airborne transmission has been suggested. The aim of our study was to investigate associations between symptoms of gastroenteritis and the presence of airborne norovirus, and to investigate the size of norovirus-carrying particles.

Methods. Air sampling was repeatedly performed close to 26 patients with norovirus infections. Samples were analyzed for norovirus RNA by reverse transcription quantitative polymerase chain reaction. The times since each patient's last episodes of vomiting and diarrhea were recorded. Size-separating aerosol particle collection was performed.

Results. Norovirus RNA was found in 21 (24%) of 86 air samples from 10 different patients. Only air samples during outbreaks, or before a succeeding outbreak, tested positive for norovirus RNA. Airborne norovirus RNA was also strongly associated with a shorter time period since the last vomiting episode (odds ratio 8.1; $P = .04$ within 3 hours since the last vomiting episode). The concentrations of airborne norovirus ranged from 5–215 copies/m^3, and detectable amounts of norovirus RNA were found in particles <0.95 μm and >4.51 μm.

Conclusions. The results suggest that recent vomiting is the major source of airborne norovirus and imply a connection between airborne norovirus and outbreaks. The presence of norovirus RNA in submicrometre particles indicates that airborne transmission can be an important transmission route.

Keywords. norovirus; airborne transmission; vomiting; hospital; bioaerosol.

Norovirus (NoV) is the major cause of viral gastroenteritis worldwide, with more than 600 million cases annually [1]. NoV is efficiently transmitted due to a low infectious dose, high viral loads in feces and vomit, and high stability in the environment. Hence, it often gives rise to outbreaks, especially in semi-enclosed settings [2]. In health-care facilities, NoV outbreaks are notoriously difficult to control and cause severe workflow disruptions, substantial economic costs, and excess morbidity [3, 4].

The main routes of NoV transmission are ingestion of contaminated water or food and contact with contaminated surfaces or infected persons [5], but some evidence also suggests that norovirus may be transmitted through the air. Hypothetically, infectious particles could be aerosolized from, for instance, vomit or toilet flushing, deposited in the upper respiratory tract during inhalation, and swallowed [6–9]. If this occurs at a significant scale, additional infection control measures may be required to prevent transmission effectively. A few outbreak reports have described occurrences where air was considered the most likely pathway for transfer [10–13]. Recently, 2 studies presented evidence of NoV in hospital air during outbreaks, detecting NoV RNA in dust and air samples [14, 15]. Bonifait et al [15] also showed that murine noroviruses remain infectious after aerosolization and that the concentration of airborne NoV may suffice to cause infections.

None of these studies provided any information on possible sources of airborne NoV or connections to outbreaks or transmission of infections. Nor did they provide any information about the size of the aerosol particles that contain viruses. Size is important, as it is the major factor determining the residence time of the aerosol particles in air and their probability of being inhaled. Aerosol particles smaller than 10 μm can be suspended in air for several hours, be transported long distances in air currents, and easily be inhaled [16].

This study aimed to investigate symptoms of gastroenteritis (ie, vomiting, diarrhea) as possible sources of airborne NoV,

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and to determine whether there is any connection between airborne NoV and outbreaks in hospital wards. In addition, the size of NoV-carrying particles was investigated.

METHODS

Study Design

Air sampling was repeatedly performed in the proximity of hospitalized patients with symptomatic norovirus infections to investigate factors associated with the presence of airborne NoV.

Setting and Participants

Air samples were collected at 3 hospitals in southern Sweden between March 2017 and May 2018. The samples were collected from areas close to patients who had suspected NoV gastroenteritis with ongoing symptoms (diarrhea and/or vomiting within the prior 24 hours). Only patients with laboratory-confirmed NoV infections were included in the final analysis.

Clinical data on symptom onset, time since last diarrhea, and vomiting within the room were collected directly from the patients when possible, and confirmed by nursing staff or medical records. Data on outbreak development were followed by close contact with the ward staff and infection control team. We defined outbreaks as the occurrence of 2 or more patients with confirmed NoV infection at the ward, including at least 1 patient with a probable ward-acquired infection. NoV-infected patients not involved in outbreaks were defined as sporadic. The ventilation systems in the patient rooms were specified for approximately 2 total air changes per hour. Patient rooms typically had most of the exhaust air extracted from the adjacent toilet.

Air Sampling

For each patient, air samples were obtained at 3 locations, in the following order: ward corridor within 5 meters from the door to the patient's room, inside the room at a distance of 2–3 meters from the patient, and in the toilet room. All toilets were directly connected to the patient's room. When feasible, air sampling was repeated within 24 hours. After sampling in the proximity of the patients, samples were also collected from the same building but outside the implicated wards, and used as negative controls.

Air samples were collected with a high-airflow liquid cyclone, Coriolis μ (Bertin Instruments), operated at 200 L/min for 10 min, with single-use collection vials containing 15 mL of phosphate-buffered saline. The lower cutoff size (d_{50}) for sampling with the Coriolis is ~1 μm [17].

The size characterization of aerosol particles containing NoV was carried out in the ward corridors on 4 occasions during outbreaks. Size-separated particle collection was performed by a cascade impactor (Next Generation Impactor, Copley Scientific) with 8 size fractions; >8.13 μm, 4.51–8.13 μm, 2.85–4.51 μm, 1.67–2.85 μm, 0.95–1.67 μm, 0.56–0.95 μm, 0.34–0.56 μm, and 0.14–0.34 μm, at a flow rate of 60 L/min for 17–120 hours [18]. The collection plates were sprayed with grease (Collection Substrate Spray, Dekati Ltd.) to avoid particle bounce-off.

Sample Analysis

NoV infections in patients were confirmed as the causative agent by reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis of fecal or vomit samples from all patients with suspected NoV gastroenteritis.

The air samples collected with the liquid cyclone (Coriolis μ) were stored at -20°C until analysis. Samples were concentrated using Amicon Ultra-15 centrifugal filter units (50 kDa cutoff, Merck Millipore KGaA) to a final volume of 200 μl. The cascade impactor samples were extracted by thoroughly swabbing each of the 8 particle size–specific collection plates with a wetted flocked swab (Copan Scientific), and then were transferred to 1 mL universal transport media (Copan Scientific).

Both patient samples and air samples were analyzed at the Department of Clinical Microbiology, Lund University, by RT-qPCR for the detection of NoV genogroups I and II, as described in Kageyama et al [19]. The lower limit of detection was 20 copies/mL sample.

Quantification was performed by comparing RT-qPCR cycle thresholds with serial 10-fold dilutions of a standard solution of NoV genogroup II (GII) (ATCC VR-3235SD). Genotyping of positive findings was performed by semi-nested sequencing of the NoV genogroup II ORF1/ORF2 junction, using the G2FB-1, G2FB-2, G2FB-3, G2SKR, and GIIFBN primers [19–21]. The genotypes were assigned according to phylogenetic analysis (https://www.rivm.nl/mpf/typingtool/norovirus/) [22].

Statistical Analysis

The Chi-square test and Fischer's exact test were used to assess crude differences between categorical variables for positive air samples in all samples and all patients separately. Using logistic regression, we assessed the association between positive air samples and time since last symptom of disease (vomiting and diarrhea), grouped in ordered time periods (0–3 hours, >3–6 hours, >6–24 hours, and >24 hours). A random-effects model was used to account for dependency between samples from the same individuals in all regression analyses [23]. The associations between positive air samples and other variables, including age (continuous), sex, room size, virus genogroup and time since onset of disease (grouped as 0–12 hours, 12–48 hours, >48 hours), were analyzed in univariate models. The same variables were also evaluated as potential confounders of any association between positive air and time since last symptom of disease in bivariate models (data not shown). To ascertain any association between time since a specific patient's last symptom and the air sample, sensitivity analyses were also performed where the corridor samples were excluded.

Ethics

The study was approved by the Regional Ethical Review Board (EPN Lund 2015/51).

RESULTS

A total of 86 air samples were obtained from the proximity of 26 patients with confirmed NoV gastroenteritis at 13 different hospital wards. NoV RNA was detected in 21 of these samples from 10 different patients (Table 1). The median number of air samples per patient was 3 (range 1–8). All 13 control samples from outside the implicated wards tested negative for NoV.

Positive Air Samples During Norovirus Outbreaks

Positive air samples were almost exclusively found during outbreaks. Of the 26 patients, 15 were part of 12 separate outbreaks, while the remaining 11 patients were sporadic cases. At least 1 NoV-positive air sample was obtained during 9 of the 12 investigated outbreaks. Samples from the ward corridor were positive in 7 of 12 outbreak wards, including 3 collected by the cascade impactor. The outbreaks involved 2–5 patients with hospital-acquired NoV gastroenteritis. None of the air samples obtained in the proximity of the 11 sporadic NoV patients were positive, except 1 where an outbreak emerged 3 days later. Patients with a positive air sample are shown in Supplementary Table 1.

Positive Air Samples in Relation to Symptoms

The detection of NoV RNA in the air was associated with a shorter period of time since the last vomiting episode ($P < .01$). This association remained after controlling for diarrhea during the same periods ($P = .01$). Of 14 air samples collected within 3 hours since the last vomiting episode, 9 (64%) were NoV positive (Figure 1). At least 1 air sample was positive from 4 of 5 patients (80%) with recent vomiting (Supplementary Table 2).

A vomiting episode within the last 3 hours was strongly associated with a positive air sample (odds ratio 8.1; $P < .01$). This was also the case in the sensitivity analysis, in which only samples from the patient's room and toilet were included (Figure 2). No obvious associations were seen between positive air samples and time period since the last instance of diarrhea ($P = .20$). Controlling for age, sex, time since onset of disease, or room size had only minor effects on the associations; these variables were not considered confounders of the norovirus-time association (data not shown).

Quantification of Airborne Norovirus

The concentration of airborne NoV RNA collected with the liquid cyclone ranged from 5–215 NoV copies/m^3, with a mean of 31 copies/m^3. The sample with the highest concentration was collected 25 minutes after a diaper was changed on a

Table 1. Characteristics of Patients and Air Samples (Liquid Cyclone), Including All and Positive Samples

	Patients, n (%)	Patients With NoV-Positive Air Sample, n (%)	*P* Value	Air Samples, n (%)	NoV-Positive Air Samples, n (%)	*P* Value
Total	26	10 (38%)	…	86	21 (24%)	…
Age group						
<80	13	3 (23%)	.11[a]	40	7 (18%)	.16[a]
≥80	13	7 (54%)	…	46	14 (30%)	…
Sex						
Male	13	4 (30%)	.42[a]	43	8 (19%)	.21[a]
Female	13	6 (46%)	…	43	13 (30%)	…
Outbreak						
Yes	15	9 (60%)	.01[b]	52	17 (33%)	.03[a]
No	11	1[c] (9%)	…	34	4[c] (12%)	…
Norovirus genotype						
II	25	10 (40%)	.62[b]	82	21 (26%)	.24[a]
I	1	0 (0%)	…	4	0 (0%)	…
Sampling site						
Corridor	21	5 (24%)	.82[a]	27	5 (19%)	.65[a]
Room	25	7 (28%)	…	39	10 (26%)	…
Toilet	15	5 (33%)	…	20	6 (30%)	…
Patients/room[d]						
1	15	5 (33%)	.61[b]	37	6 (16%)	.04[a]
2	7	4 (57%)	…	16	8 (50%)	…
3–4	3	1 (33%)	…	6	2 (33%)	…

Abbreviation: NoV, norovirus.

[a]Chi-square test for differences in positive air samples within categories.

[b]Fischer's exact test for differences in positive air samples within categories.

[c]No outbreak at the time of sampling, but emerged 3 days later.

[d]Total number of patients at the room of the norovirus positive patient.

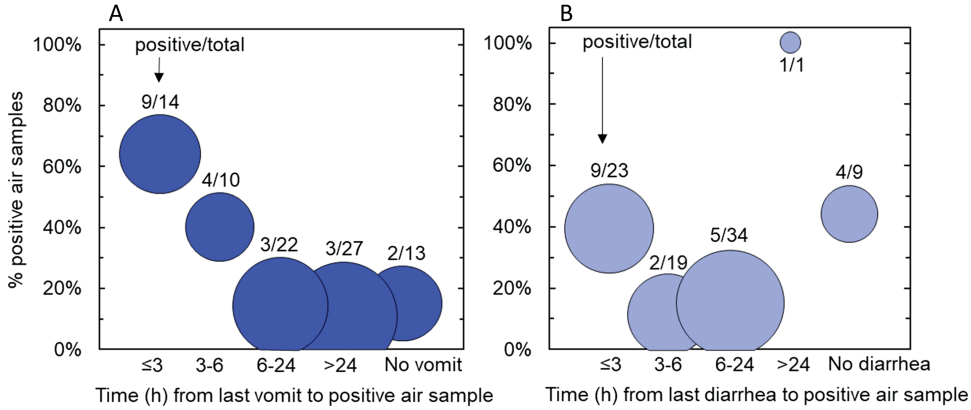

Figure 1. Percent of NoV-positive air samples in relation to time since (*A*) last vomiting episode and (*B*) last diarrhea. The area of each bubble is proportional to the total number of air samples within each time interval. The values above each bubble represent the number of positive and total air samples. Abbreviation: NoV, norovirus.

patient with no previous record of any vomiting in the room (Supplementary Table 1, Case 4).

Size of Particles Containing Norovirus RNA

The size-separating cascade impactor results showed NoV-positive samples in 3 of 4 investigated outbreaks (Figure 3). Positive samples were found in aerosol particle size fractions >4.51 µm and <0.95 µm; however, the size fractions that were positive differed between the 3 outbreaks. No difference in virus concentrations between small and large size fractions was observed.

Norovirus Genotypes

Of the 26 patients included, 25 were infected by NoV genogroup II, of which 20 were genotype GII.4 Sydney and 1 was GII.6. Sequencing failed in 3 patients. There was 1 patient who was infected with NoV genogroup I. Genotyping was successful in 2 air samples obtained at 2 different wards. In both cases, genotype GII.4 Sydney was detected, with sequences identical to the patients' fecal sample strains.

DISCUSSION

This study confirms the airborne dispersal of NoV RNA, links airborne NoV to patients' symptoms, and provides new information on particle size, thus establishing the transmission ability of aerosol particles containing NoV. The results also suggest vomiting as the major source of airborne NoV and imply a connection between airborne NoV and outbreaks.

Several case studies have suggested airborne dispersal of NoV as a cause for outbreaks [10–13], but only 2 previous studies reported evidence of NoV in the air during outbreaks in hospitals. Nenonen et al [14] detected NoV in airborne particles collected in ionizer traps and Bonifait et al [15] detected widespread airborne NoV in patient rooms and other ward areas, partially using the same air collection methodology as in the

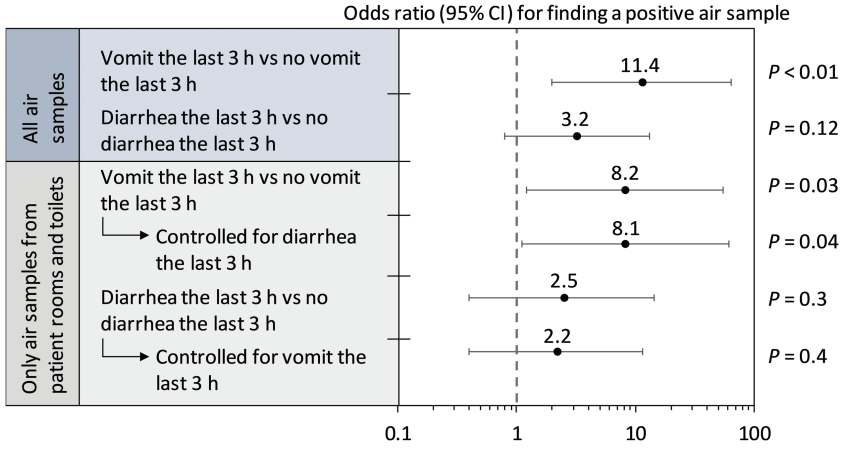

Figure 2. Odds ratios for positive air samples within 3 hours from last symptom versus no symptom within 3 hours. The sensitivity analysis was performed using logistic regression with a random effects model to account for dependency between samples from the same individuals. Abbreviation: CI, confidence interval.

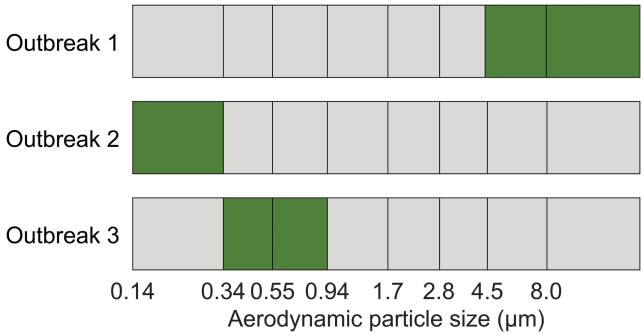

Figure 3. Size ranges of norovirus-positive aerosol samples (dark green) from the cascade impactor during 3 different outbreaks (negative stages are colored gray). During the fourth outbreak, all stages were negative. Note that the width of the stages is on a log scale.

present study. Our study confirms that airborne NoV RNA is common during outbreaks, also in open ward areas.

The present study also included sporadic cases with NoV infection, but almost no positive air samples were found in connection to this group. In fact, at the ward where the only sporadic case with positive air samples was treated, an outbreak emerged a few days later. This case, together with the high prevalence of positive air samples in outbreaks compared to sporadic cases, suggests that airborne dispersal could be an important factor in NoV outbreak developments in health-care settings. A previous study showed that vomiting during NoV infection is a risk factor for outbreak development [24]. The present study supplies additional evidence that vomiting may be a risk factor for further transmission and outbreaks.

We found a strong association between positive air samples and time since last vomiting episode, inferring that vomiting was the major source of airborne NoV. Even if the association between time since last diarrhea episode and NoV-positive air did not reach significance in the present study, diarrhea and toilet flushing may still generate aerosols containing NoV. Interestingly, the air in the proximity of 1 of the cases was positive for NoV even though no symptom had yet occurred in the room, implying other potential sources of airborne NoV, such as emissions from contaminated clothes or exhaled air [25, 26].

Norovirus is acknowledged to spread by splashing droplets that are generated from the patient, as during vomiting. During this process, large numbers of small droplets are also produced that are likely to evaporate to dry droplet nuclei, which have a low settling velocity and, thus, can stay airborne for several hours [16, 27]. Although the results from the size-separated collection varied in the 4 investigated outbreaks, an important conclusion is that airborne particles as small as <1 μm contain detectable amounts of NoV. Particles in this size range are easily inhaled and have a typical probability of 10–30% to deposit in the mouth, nose, or conducting airways, from where they are likely to be transported to the gastrointestinal tract.

The concentration of airborne NoV in our study was in the same range as previously reported [15]. Theoretically, given the highest virus concentration measured, the 50% human infectious dose of 20 to 1000 copies would be reached after 10 minutes to 8 hours of normal breathing [28–30]. If the airborne genomes represent viable viruses that reach the gastrointestinal tract after inhalation, there is a high likelihood that airborne NoV can cause disease. Future studies combining environmental sampling of NoV with novel culture methods can provide decisive information regarding the infectivity of airborne NoV [31].

The method of sampling directly into liquid with a cyclone is favorable, since collection is fast, the air sample volume is large (2 m^3), and extraction is easily performed. There are collection methods with better recovery, but they are less practical in clinical settings [32]. Collection with a liquid cyclone sampler may carry a risk for cross-contamination within the air inlet, but since all control samples in our study were negative, significant contamination of the samples was considered unlikely. The cascade impactor sampler has a high sampling efficiency (>95%) within the defined particle size fractions; however, the equipment is bulky and noisy and, thus, could only be used in some of the outbreaks and not in the patients' rooms.

Due to the rapid course of NoV gastroenteritis, sampling shortly after the initial symptoms appear is difficult. As symptoms may cease before the NoV infection is confirmed by laboratory analysis, we mainly sampled air from suspected cases and later excluded patients without NoV infection. Sampling earlier on in the clinical course was easier during outbreaks, as more patients became ill successively. This may have biased our data to sampling sooner after symptoms during outbreaks.

Even though the wards were visited within hours of confirmed or suspected disease, collecting the information about the exact time of a diarrhea and vomiting episode from patients or staff was sometimes challenging. The connection between an air sample and an individual could also be difficult in corridors and rooms with 2 or more patients. In those situations, we linked the air samples to the patient with the most recent symptom. As this primarily affected the corridor samples, statistical sensitivity analyses excluding these samples were conducted.

Our study is the largest study of airborne NoV, but was still not large enough to conduct a multivariable analysis controlling for more than 1 confounding factor. Nevertheless, we believe that the main findings are valid and generalizable. Still, conclusions regarding possible sources of airborne NoV RNA are based on statistical associations and not direct evidence of causation.

Face masks have since long been advocated as personal protective equipment during the care of vomiting patients [33]. The finding of airborne NoV in sufficient concentrations to cause

disease raises the question of whether and how to optimize infection control efforts and personal protection equipment. Further studies are warranted before generally recommending full airborne precautions, including respirators and negative pressure rooms, during care of patients infected with NoV. In view of possible transmission by air, hospital staff and planners should take airflow and ventilation rates into consideration to better prevent this contagious virus. Closed doors would be a simple step to reduce dispersal into neighboring rooms, but this alone is not sufficient: doors were usually closed during our visits to the implicated wards. When possible, efforts to reduce vomiting can also reduce aerosolization of NoV.

In conclusion, this study provides additional evidence that airborne NoV can be present in concentrations that are high enough to cause infections, and in particle size fractions that remain airborne for long periods and are easily inhaled. We present unique data indicating a strong association between vomiting and the presence of airborne NoV RNA. The observed connection between airborne NoV RNA and outbreaks supports the hypothesis that the airborne transmission route may be of importance.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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