Nosocomial Transmission of Norovirus Is Mainly Caused by Symptomatic Cases

Faizel H. A. Sukhrie,1,2 Peter Teunis,3,4 Harry Vennema,1 Cedrick Copra,2 Matthias F. C. Thijs Beersma,2 Jolanda Bogerman,2,5 and Marion Koopmans1,2

1Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control (RIVM), Bilthoven; 2Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands; 3Epidemiology and Surveillance Unit, Centre for Infectious Disease Control (RIVM) Bilthoven; 4Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia; and 5Municipal Health Service, Rotterdam-Rijnmond, The Netherlands

Background. Nosocomial norovirus (NoV) infection is common and may increase the burden of disease in healthcare settings, particularly in vulnerable hospitalized patients. Implementing effective infection control during and after admission may limit further spread, but evidence-based measures are lacking.

Methods. In this study, we performed a systematic evaluation of sources and modes of transmission during NoV outbreaks within 2 types of healthcare facilities. An outbreak protocol was developed to sample all patients and healthcare workers (HCWs) with and without symptoms on wards involved in outbreaks. Data on clinical history and possible high-risk exposures were collected. Five outbreaks were investigated, involving 28 patients with recognized symptomatic NoV infection.

Results. Enhanced sampling, however, yielded 65 additional cases, of whom 14% (n = 9) were asymptomatic patients, 57% (n = 37) were symptomatic HCWs, and 17% (n = 11) were asymptomatic HCWs. For 12% (n = 8), clinical data were not provided (2 HCWs and 6 patients). On the basis of the shedding kinetics, the onset of infection was estimated for each case. The generation interval was then used to construct plausible transmission pathways and reproduction numbers for symptomatic and asymptomatic patients and HCWs.

Conclusions. We found that symptomatic patients and HCWs were more often involved in transmission events than asymptomatic shedding. Asymptomatic HCWs rarely contributed to transmission, despite high levels of fecal virus shedding.

Norovirus (NoV) is the most common cause of acute gastroenteritis worldwide [1]. Transmission of NoV most often occurs through direct contact with NoV shedders, or indirectly through environmental or food contamination with human feces or vomit, especially in closed settings such as hospitals, nursing homes, cruise ships, and hotels [2–4]. In a previous study, we demonstrated the high transmissibility of NoV in a large tertiary care hospital, resulting in frequent nosocomial outbreaks (OBs) [5]. Transmission from chronic patients shedding NoV for a long period of time may also occur, as we have demonstrated previously [6].

There are few examples of contribution of asymptomatic shedders to (nosocomial) OBs [7, 8], although several publications have reported the high prevalence of asymptomatic shedding [9–12]. In adult volunteer studies, 32% (13 of 41) of infected persons remained asymptomatic after challenge with Norwalk virus [13, 14], but this involves persons with good general health and a rare genotype, not representative for the hospitalized group of patients. Systematic studies addressing the role of asymptomatic carriage of NoV and its implications in these settings are lacking. Implementation of stringent infection control measures is recommended to control OBs, but scientific evidence underpinning these measures is missing [15]. One of the most frequently asked questions is when an infected healthcare worker (HCW) can return to work, particularly because people may shed NoV for
METHODS

We developed an enhanced OB investigation protocol focusing on the identification of possible sources and modes of transmission of NoV within a tertiary care hospital and 2 nursing homes in the region of Rotterdam (Netherlands) between January 2009 and March 2010. Directors or chiefs from centers reporting 2 or more cases of NoV confirmed by polymerase chain reaction (PCR) within the same ward within the same week were asked to participate in this study. The study protocol included random sampling irrespective of symptoms of all patients and HCWs on affected wards using a strict protocol as is done for methicillin-resistant Staphylococcus aureus [18]. Persons involved in the OB who met the inclusion criteria of the study protocol were tested weekly until a negative test was returned. Measured virus concentrations (cycle threshold [CT] measurements) in the fecal samples were used to calculate the most probable first day of shedding by means of regression analysis. Details are provided in the “Appendix.” With the onsets of shedding known for all sampled subjects, serial intervals between all pairs of infected subjects could be calculated. Subsequently, we computed the probability of transmission between any pair of cases by using the distribution of serial intervals as described previously [19]. With this transmission matrix, reproduction numbers were estimated for symptomatic and asymptomatic patients and HCW.

The study was done in 1 academic hospital, and 2 nursing homes covered by the municipal health service of Rotterdam-Rijnmond. An OB was defined as 2 or more cases of patients or HCW in the same department with gastroenteritis symptoms within 24 hours. Gastroenteritis was defined as diarrhea and/or vomiting (2 or more episodes within 24 hours) that could not be attributed to underlying illness or medication.

The duration of each OB was defined as the period starting 7 days before the start of symptoms of the index case and lasting until 7 days after the start of symptoms of the last case. For this period, all patients and HCWs involved with the care and treatment of patients, or using a shared toilet in the treatment facility or healthcare unit, were approached for participation. Patients who had been discharged within the 7 days before the onset of illness in the first recognized case were not approached. Children up to 18 years of age and participants with a legal representative could be included in the study after authorization of their parents and/or legal representative.

Each person who consented to participation in this study was provided with a stool sampling kit and a small questionnaire aimed to consider their clinical state. Quantitative reverse transcription–PCR and genotyping of the strains were performed as described previously [5]. Persons with NoV-positive stool specimens were asked to resend a specimen once a week until they tested negative.

Data Analysis

Estimation of Onset of Excretion in Asymptomatic Cases
To calculate likely transmission patterns, an estimate of the onset of infectiousness is needed for each case. In previous studies using only symptomatic cases, this has been assumed to be the date of illness onset [19], but this approach cannot be used for the asymptomatic shedders. Therefore, we estimated the first day of shedding from the time course (increase followed by decrease) of virus excretion using stool sample data that were available for both asymptomatic and symptomatic cases. A parametric model of virus shedding was used based on a compartment model of virus production and excretion in the intestinal tract (“Appendix”). After transformation to −log scale (to accommodate for CT measurements as obtained by real-time PCR analysis), the model was fitted in a 2-level Bayesian framework, allowing for variation in time course (rates of increase and decrease) and amplitude (peak level of virus excretion) among individual shedders. Onset of shedding, measured as time from the first sample, was included as a covariate that could be estimated for both symptomatic and asymptomatic shedders. More details of the model and parameter estimation are available in the “Appendix.”

Transmission Patterns

With the estimated onsets of shedding, asymptomatic cases could be added to the dataset, extending the epidemic curve. With the help of the serial interval distribution (defined as the distribution of time between onsets of shedding in successive symptomatic or asymptomatic cases), the onset dates may then be used to estimate the likelihood of transmission between any pairs of cases, as reported previously [20]. Different OBs were treated as separate (isolated) clusters, and no transmission was assumed to occur between cases infected with different genotypes. In contrast to patients, the HCWs cannot be assumed to be in continuous contact with hospitalized patients or nursing
home residents. To adjust for contact time, the transmission probabilities of HCWs were weighted by the fraction of the time they were present (typically 8 of 24 hours). No difference in prior weighting was applied for symptomatic/asymptomatic cases.

Pairwise probabilities of transmission (the elements of the transmission matrix) were estimated by Markov chain Monte Carlo sampling, as reported previously [19], resulting in a Monte Carlo sample of matrices with elements representing the probabilities of transmission from any subject (i) to any other subject (j).

Different iterations of this matrix were used to obtain uncertainty estimates for any of the selected output metrics. Initial parameter values for the serial interval distribution were taken from a previous study [19]: a gamma distribution with parameters (shape parameter 3.35, scale parameter 1.09, leading to a mean serial interval of 3.6 days, and mode [most likely interval] 2.6 days). As soon as convergence was reached, adjustment of the serial interval distribution was allowed by updating its parameters, followed by optimization of the transmission matrix until (again) convergence. This nested approach resulted in improved estimates (higher posterior probability) with slightly shorter estimates of the serial interval compared with its initial value.

**Transmission by Category**

The number of infections caused by any subject i was estimated by summing over all cases j the probability that j was infected by i [20]. This is defined as the reproduction number of subject i. Thus, transmission among and between patients and HCWs, and from patients to HCWs and the reverse, were calculated as reproduction numbers of these categories for each of the OBs. We further stratified the analysis by type of symptoms, differentiating between cases with diarrhea and cases with vomiting.

**RESULTS**

**Outbreak Analysis**

From January 2009 until March 2010, 5 OBs were reported caused by 3 different NoV genotypes (ie, GII.4, GII.2, and GII.7). One cluster (OB 1) was segregated by use of molecular typing into 2 groups of patients infected with GII.4 2008 (n = 18) and GII.4 2006b (n = 4), respectively (Table 1). Because the GII.b strain sequences were diverse, these patients were excluded from the group as likely new introductions unrelated to the OB. Full details of this OB will be published elsewhere. All of the other OBs consisted of a single genotype. The duration of the OB ranged from 38 days to 77 days.

OBs 1, 2, and 3 were detected in the hospital, whereas 4 and 5 occurred in nursing homes. In total, 386 HCWs and 127 patients/residents were approached for participation, and 257 (66%) of the HCWs (range 60%–72%) and 79 (77%) of the patients/residents (range 68%–100%) agreed to take part in the

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Abbreviations: HCW, healthcare worker; OB, outbreaks; neg, negative; P, patient or resident; PCR, polymerase chain reaction; pos, positive.

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study. In total, 50 (20%) of participating HCWs had at least 1 positive stool (PCR), and 43 (54%) of participating patients tested positive. Details on testing results are listed in Table 1. The enhanced case finding resulted in a 276% (n = 69) increase in the total number of subjects with confirmed NoV infection, assuming that typically only symptomatic NoV in patients/residents are reported, as has been detected in the routine diagnostics. The shedders that were symptomatic detected in each OB were as follows: OB 1 (n = 16), OB 2 (n = 6), OB 3 (n = 8), OB 4 (n = 18), and OB 5 (n = 17). Of the additionally diagnosed shedders, 29% (n = 20) did not report any symptom.

**Modeling**

The analyses described below were done only for the GII.4 OB (OBs 1, 4, and 5), because transmission patterns may differ for viruses belonging to different genotypes, thus precluding pooling of all data [5, 6]. GII.4 strains are predominant in OBs in healthcare settings [21]. The same serial interval distribution was used for all OBs because there is no information at this point about the heterogeneity in serial intervals among NoV GII.4 variants. The shedding kinetics of all the cases involved in the GII.4 OB was used to estimate onsets of shedding. Transmission analysis produced adjusted estimates of the serial interval distribution and estimates of the probability of transmission between any pairs of cases in the studied OB. An example of a transmission tree derived from the transmission matrix iteration with the highest posterior probability (as approximation of the posterior mode) is shown in Figure 1. The Monte Carlo estimates for the probability of transmissions by any infected subject were used to calculate their individual reproduction numbers and these could be averaged by category, stratifying cases by symptom status for NoV-infected persons for all cases without diarrhea (white boxes; Figure 2, left panel); this resulted in an average reproduction number of 0.85 (95% confidence interval [CI], .55–1.05). For all cases with diarrhea (gray boxes; Figure 2, left panel), this resulted in an average reproduction number of 1.64 (95% CI, 1.56–1.70). This analysis shows that the contribution of asymptomatic shedders to transmission was significantly lower than that of symptomatic individuals when data from all OBs were combined, although the pattern differed between OBs: in OB 4, the difference in contribution to transmission from asymptomatic cases was not significantly lower. Next, we stratified the data for patients and HCW (Figure 2, right panel). For the symptomatic patients, the stratification resulted in an average reproduction number of 1.89 (95% CI, 1.71–2.12). For the symptomatic HCWs, the stratification resulted in an average reproduction number of 1.30 (95% CI, 1.08–1.52). For the asymptomatic HCWs, no reproduction numbers could be detected. This result shows that

![figure 1](image1.png)

**Figure 1.** Example of a plausible transmission tree for one of the outbreaks (OB5), based on outcome of markov chain monte carlo as described in the “Methods” section. Patients/residents (P) and healthcare workers (H) are shown, symptomatic cases are indicated in black characters, and the asymptomatic cases are shown as gray characters.

![figure 2](image2.png)

**Figure 2.** The box plots indicate (from the center outward): median, quartiles (25 and 75 percentiles) and 95% interval (whiskers, 2.5 and 97.5 percentiles). Left: estimated reproduction numbers for each outbreak for cases with (gray) and without (white) symptoms (diarrhea). Right: estimated reproduction numbers for all outbreaks, stratified for symptomatic (gray)/asymptomatic (white) staff (healthcare workers [HCW]) and patients, with and without symptoms (only the first 5 days of all outbreaks). In outbreak 1, transmission from asymptomatic cases did not occur and no transmission was found from asymptomatic HCW, therefore these boxes are missing. Abbreviation: $R_{eff}$, reproduction numbers.
the difference was greatest for the HCWs where contribution to spread by asymptomatic persons could not be detected, and for symptomatic HCWs, the reproduction numbers were significantly lower than for symptomatic patients. Asymptomatic patients did contribute to transmission, but less than persons with symptomatic infection.

**CONCLUSIONS AND DISCUSSION**

In this study, we performed structured OB investigations with enhanced sampling after notification of a suspected OB to obtain data that can be used to design evidence-based strategies for prevention of spread of NoV in healthcare settings. We have seen that enhanced sampling yielded a 232% increase of identified shedders consisting of asymptomatic patients and HCWs, and symptomatic HCWs that normally would be missed in the routine sampling. These results illustrate the potential underestimation of the size and duration of an OB in regular OB investigations where sampling of HCWs with health complaints is not done routinely, and sampling to identify asymptomatic shedders is rare.

HCWs in general have been presumed to play a role in transmission because their work involves direct contact with many patients. We used the enhanced surveillance data to address the question of who contributes most to shedding. Persons infected with NoV may continue to shed high amounts of virus for several weeks after resolution of symptoms [5, 6, 22], and hospital hygienists or persons working in the food industry need to decide whether it is safe to return to work. The current Centers for Disease Control guideline recommends that people should not resume work until 2–3 days after clinical recovery, but evidence for this is lacking and the extra leave of absence may be problematic in small food establishments or healthcare settings during peak seasons. Therefore, we tried to determine who contributes most to the spread of infection, and whether this was related to being symptomatic.

Our findings clearly suggest that symptomatic shedders are more frequently involved in transmission than asymptomatic shedders. No transmission was observed from asymptomatic HCWs despite considerable levels of virus shedding in the stool. This shows that an infected person does not need to be infectious, most likely related to proper personal hygiene. The latter is indicated by the difference between HCWs and patients with NoV—there was a significant difference in the contribution of HCWs to transmission, both for symptomatic and asymptomatic shedders, and the awareness of HCWs of the need for hygiene would be the most likely explanation for this difference. For instance, proper hand hygiene can lower the incidence of NoV [23], although overall evidence for effects of hand hygiene on prevention of healthcare-associated infections is considered to be weak [24]. Nevertheless, our observations suggest that a more important factor is the health status. Our study did not reveal differences between persons with vomiting and persons with diarrhea in their contribution to spread of infection, as is frequently suggested. The observations may be related to the power of the study and should ideally have been addressed in more extensive studies. However, these studies are difficult to conduct because the occurrence of OBs cannot be planned, and willingness to participate in studies is influenced by the extra work needed in patient care during OBs. Based on these findings, we conclude that the period of mandatory leave from work for infected HCWs potentially may be relaxed. However, this conclusion relies on unbiased reporting of illness episodes by HCWs, which may be overly optimistic. In a study of self-compliance during a NoV OB, it was found that a quarter of affected personnel did not adhere to quarantine recommendations [25].

Illness reports from hospitalized patients and from HCWs are often done through different reporting channels (for instance, the hospital hygiene department and the office of personnel), although the combination of observations would enhance the early detection of OBs. Our study shows that notified cases in an OB may be the proverbial tip of the iceberg, and increased awareness is warranted.

Even with enhanced sampling, there is still evidence of underascertainment: of the HCWs that did agree to participate, 13% of those that tested negative for NoV did have symptoms compatible with acute NoV infection. There are several possible explanations why no virus was found. Sampling of these cases may have been too late in that virus excretion may have decreased to undetectable levels [16]. In addition, HCWs may be capable of early clearance of the virus due to their better immunity status compared with hospitalized patients [26]. Therefore, we compared the results with and without the NoV-negative HCWs with diarrhea. Adding them to the transmission analysis produced similar results for the distributions of reproduction numbers in different subject categories (as in Figure 2, results not shown).

An important factor that was not included in our study and may influence the construction of transmission trees is the role of environmental contamination. This is a potential source of protracted OBs, as has been documented in multiple OB reports [27–30]. In our approach, we disregarded this option by assuming relatively short intervals between consecutive cases, based on distribution of generation intervals observed during person-to-person OBs. In the OBs studied here, we believe that environmental persistence did not play a major role because the observed generation intervals plotted based on the dates of onset of new cases did not differ significantly from the distribution observed during a model OB [19]. Of course, this cannot be taken as a generalization because situations may differ between hospitals and healthcare settings, resulting in vast...
differences in the likelihood of environmental contamination [27, 30, 31].

To our knowledge, this is the first time estimates of the first-day shedding of asymptomatic cases with NoV have been made. Virus excretion patterns were only used for determining the onset of shedding in symptomatic and asymptomatic shedders. As a further refinement, it is tempting to use other aspects of the excretion patterns, such as duration of shedding and the peak levels of excreted virus, as proxies for the infectivity of individual cases. In a subsequent study, we plan to enhance the transmission analysis method to incorporate these additional information sources, allowing for individual differences in infectivity to improve the identification of likely transmission pairs.

To conclude, effective guidelines are needed for diagnostics that includes HCWs and contacts of positive cases (enhanced screening). Awareness of reporting symptoms by the HCWs and patients is still an important element for rapid OB detection to possibly achieve timely intervention.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes
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