

Enhanced Hygiene Measures and Norovirus Transmission during an Outbreak

Janneke C.M. Heijne,¹ Peter Teunis,¹ Gabriella Morroy, Clementine Wijkmans, Sandy Oostveen, Erwin Duizer, Mirjam Kretzschmar, and Jacco Wallinga

Control of norovirus outbreaks relies on enhanced hygiene measures, such as handwashing, surface cleaning, using disposable paper towels, and using separate toilets for sick and well persons. However, little is known about their effectiveness in limiting further spread of norovirus infections. We analyzed norovirus outbreaks in 7 camps at an international scouting jamboree in the Netherlands during 2004. Implementation of hygiene measures coincided with an 84.8% (95% predictive interval 81.2%–86.6%) reduction in reproduction number. This reduction was unexpectedly large but still below the reduction needed to contain a norovirus outbreak. Even more stringent control measures are required to break the chain of transmission of norovirus.

Gastroenteritis is one of the most common causes of illness (1). Recent findings indicate norovirus is the most common cause of gastroenteritis (2,3). Of all gastroenteritis outbreaks reported in the Netherlands during 2002, 54% were caused by norovirus (4). Norovirus is predominantly transmitted through the fecal–oral route, either indirectly through contaminated food or surfaces or directly from person to person (5). It can be transmitted through small infectious droplets (aerosols) after a vomiting episode (6,7) and can survive for a very long time in the environment (5,8). Most norovirus outbreaks are seen in settings where clusters of vulnerable, susceptible persons live closely together, such as nursing homes, hospitals, and daycare centers (4).

Author affiliations: National Institute for Public Health and the Environment, Bilthoven, the Netherlands (J.C.M. Heijne, P. Teunis, E. Duizer, M. Kretzschmar, J. Wallinga); Emory University, Atlanta, Georgia, USA (P. Teunis); Municipal Health Services “Hart voor Brabant,” ‘s-Hertogenbosch, the Netherlands (G. Morroy, C. Wijkmans, S. Oostveen); and University Medical Center Utrecht, Utrecht, the Netherlands (M. Kretzschmar, J. Wallinga)

DOI: 10.3201/1501.080299

and in settings in which turnover of susceptible persons is high, such as hotels and cruise ships (9,10).

Norovirus infection can cause serious medical complications, such as dehydration, in persons with underlying illness (11). No antiviral treatment exists for norovirus infection, and although norovirus vaccines are in development (12), none are available yet. Early studies suggested that norovirus outbreaks could be contained by rapid implementation of enhanced hygiene measures, such as washing hands, thoroughly cleaning contaminated surfaces, avoiding contact between sick and healthy persons, and requesting caretakers and cleaning staff to wear gloves and aprons (13–16). However, in nursing homes or on cruise ships, norovirus can cause consecutive outbreaks, even after implementation of strict hygiene protocols (9,17,18). No quantitative estimates exist of the results of such enhanced hygiene measures on reducing further transmission of norovirus. To our knowledge, the effect of enhanced hygiene measures has not been investigated in randomized controlled trials or in statistical analyses of outbreaks.

We investigated the effect of enhanced hygiene measures on reducing norovirus transmission during an outbreak. We measured the effectiveness of enhanced hygiene measures as the relative reduction in the reproduction number—defined as the average number of secondary cases caused by 1 typical case—in the absence of and after enhanced hygiene measures. The value of this reproduction number provides crucial information about transmission potential: if the reproduction number exceeds the threshold value of 1, the number of new cases will increase over time; if it is <1, the number of new cases will decline over time, and eventually the chain of transmission will break.

The time course of the reproduction number during an outbreak can be inferred from the epidemic curve (19,20).

¹These authors contributed equally to this article.

We obtained a detailed epidemic curve of a norovirus outbreak at an international scout jamboree in the Netherlands from July 26 through August 10, 2004. This outbreak was ideally suited to estimating the effects of enhanced hygiene measures because the date enhanced hygiene measures began was recorded. Moreover, because the scouts were divided into 7 camps, the jamboree provided a natural experiment in which the camps could be regarded as “experimental units,” with varying durations between introduction of the virus and implementation of enhanced hygiene measures.

Methods

Data

An outbreak of norovirus infection occurred at an international scout jamboree in the Netherlands during the summer of 2004 (21). Approximately 4,500 persons from 32 countries attended this event. At the start of the scout jamboree on July 26, 2004, two participants became ill with symptoms of gastroenteritis. The outbreak affected at least 326 persons with typical, generally mild symptoms of gastroenteritis (case-patients). Most ill persons experienced vomiting (258) and/or diarrhea (195). Ninety-two ill persons visited a local first aid tent; another 54 were admitted to a local hospital for rehydration.

The jamboree was held on a large site, $\approx 600 \text{ m} \times 1,000 \text{ m}$. Jamboree participants were divided into 7 camps according to age: 3 camps each for participants 11–14 and 15–17 years of age and 1 camp for staff ≥ 18 years of age. The 7 camps were situated around a central field for joint activities; most activities were organized within the camps. The camps were labeled A–G, according to the day the first participants became sick. For 296 (91%) of 326 case-patients, the camp label was known (Figure 1, Table).

On July 29 (day 3 of the jamboree), the Municipal Health Service “Hart voor Brabant” in ’s-Hertogenbosch provided advice on enhanced hygiene measures (22), instructed participants about proper hand hygiene and use of soap pumps and disposable paper towels, and assigned separate toilets for sick participants. In addition, the Municipal Health Service provided guidelines for cleaning toilets and contaminated surfaces with a 1,000-ppm chlorine solution. Sick participants were instructed to go to a first aid tent. Sick participants were not allowed to prepare food until 3 days after their last symptoms. Persons working in the jamboree’s field hospital were instructed to wear gloves, aprons, and surgical masks and to minimize the number of patients per nurse. The scout jamboree ended on August 5.

Norovirus was epidemiologically implicated as the causative agent (21) of the outbreak and was confirmed in stool samples through a standard reverse transcription-PCR protocol (23). Typing of 7 samples from case-patients in

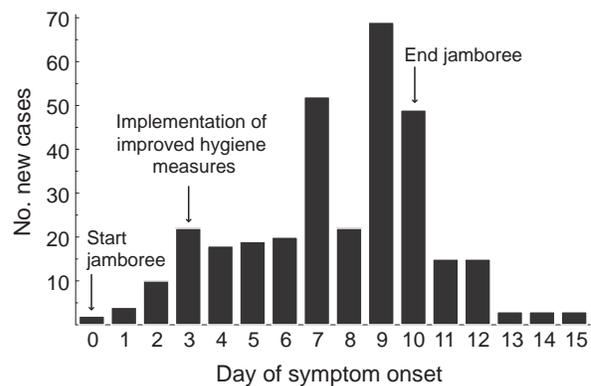


Figure 1. Epidemic curve of an outbreak of norovirus at an international scout jamboree in the Netherlands, starting July 26, 2004 (day 0).

whom symptoms first developed 7–9 days after the outbreak began resulted in 3 norovirus genotypes: 2 samples typed as norovirus genotype GI.4, 1 sample typed as genotype GI.5, and 4 samples typed as genotype GII.4–2004. We did not detect any multiple infections.

During the outbreak, the Municipal Health Service assessed the number of new cases from typical gastroenteritis symptoms self-reported by participants and staff. After the jamboree, participants and staff were given a questionnaire asking them to report to the Municipal Health Service whether gastroenteritis had developed within a week after departure. The questionnaire asked the date of symptom onset, symptoms, camp label, and hospital admission.

Reproduction Number

Estimation of Reproduction Numbers

We estimated the reproduction number for every case during the norovirus outbreak at the jamboree. Using the date of symptom onset for each case, we applied statistical methods to reconstruct likely patterns of who infected whom (online Technical Appendix 1, available from www.cdc.gov/EID/content/15/1/24-Techapp1.pdf). We first calculated the difference in day of symptom onset for all combinations of case pairs. To calculate the probability of transmission between any pair of cases, we needed information from the distribution of generation times (defined as the time between day of symptom onset in a secondary case and day of onset in its primary case) (19,24). To estimate the generation time distribution for norovirus infections, we used observations of generation times from several large norovirus outbreaks in child daycare centers in Sweden during 1999 (25). These generation times were well described by a gamma distribution (Figure 2), for which the parameters were estimated by the method of maximum likelihood (online Technical Appendix 1). The frequency

RESEARCH

Table. New norovirus cases during outbreak at international scout jamboree, the Netherlands, starting on Jul 26, 2004 (day 0), by day of symptom onset and camp label

Day of onset	Camp, no. new cases/d							Unknown	Total (n = 4,500)
	A (n = 485)	B (n = 721)	C (n = 729)	D (n = 499)	E (n = 735)	F (n = 825*)	G (n = 506)		
0	1	1	0	0	0	0	0	0	2
1	1	0	1	0	0	0	0	2	4
2	0	2	2	1	1	3	0	1	10
3	2	7	9	0	2	1	0	1	22
4	3	4	2	1	2	2	4	0	18
5	0	10	1	2	1	1	2	2	19
6	0	12	3	2	2	0	0	1	20
7	2	19	14	2	3	3	6	3	52
8	3	5	8	2	2	1	1	0	22
9	7	10	14	0	2	10	24	2	69
10	5	4	3	2	0	16	8	11	49
11	3	2	4	1	0	1	1	3	15
12	4	1	4	2	1	1	0	2	15
13	0	0	1	0	0	0	1	1	3
14	0	0	1	0	0	1	0	1	3
15	0	0	1	0	1	1	0	0	3
Total†	31	77	68	15	17	41	47	30	326

*This number is estimated.

†Overall attack rate: 7.2. Attack rate by camp: A, 6.4; B, 10.7; C, 9.3; D, 3.0; E, 2.3; F, 5.0; G, 9.3.

distribution of generation times was used to assign a likelihood of transmission for any pair of cases, allowing estimation of the transmission probabilities. We then used a powerful statistical sampling algorithm to generate a large sample of plausible transmission patterns (for technical details, see online Technical Appendix 1). The expected value of the reproduction number for a specific case was the sum of all transmission probabilities of outgoing infectious contacts to all other cases in the outbreak. For cases in which symptoms began the same day, we calculated the mean minimum and maximum values of the reproduction number. For the entire sample of transmission probabilities, we obtained the 0.025 and 0.975 quantiles for these 3 metrics as predictive intervals.

Host Population Structure and Pathogen Genotype

We incorporated additional information about the camp label of almost all case-patients and the pathogen genotype for 7 case-patients into the estimation procedure by adding a "weight" to the transmission probabilities between pairs of cases. Here we considered 2 extreme cases for mixing between camps. The first extreme case was homogeneous mixing between all participants of the jamboree, as we assumed in the analysis described above; to achieve this, we assigned a weight of 1 to any pair of cases. The second extreme case was mixing within camps only and no mixing between camps. In this instance, the transmission probabilities for pairs of case-patients that stayed in different camps were assigned a weight of 0, and the transmission probabilities for pairs of case-patients that stayed in the same camp were given a weight of 1. The transmission probabili-

ties for pairs of cases with known different genotypes were assigned a weight of 0, and the transmission probabilities for pairs of cases with known identical genotypes were assigned a weight of 1.

Expected Time Course of Reproduction Number

If the enhanced hygiene measures resulted in a sudden decline in transmission, the expected decline of the reproduction number would be gradual. Four factors determined the expected time course: the day enhanced hygiene measures began, the cumulative frequency distribution of generation times, the reproduction number without enhanced hygiene measures, and the relative reduction of the reproduction number attributed to hygiene measures. We express the reproduction number as a function of these 4 factors (online Technical Appendix 1) and fitted this function to every sampled time course of the mean reproduction number for days 0–5, with least squares regression to obtain point estimates and 95% predictive intervals for the parameters describing the reproduction number in the absence of hygiene measures and relative reduction of the reproduction number resulting from the hygiene measures.

Testing of the Estimation Procedures

We tested the estimation procedure by simulating epidemic curves with known reproduction numbers. The interval estimates for reproduction numbers covered the actual values for days 0–7. We detected a slight downward bias for the estimated value of reproduction numbers and a slight downward bias for the estimated relative reduction of reproduction numbers after implementation of

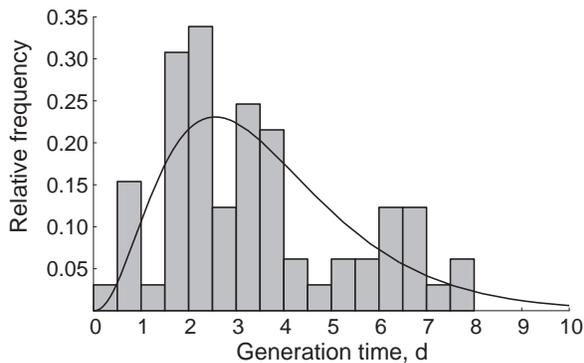


Figure 2. Generation time distribution for norovirus infections. Generation time is the time between onset of symptoms in successive case-patients. The histogram gives the relative frequency in norovirus outbreaks in Sweden in 1999 (25); the black line indicates the maximum-likelihood fit of the gamma distribution.

enhanced hygiene measures, indicating that the values obtained by the estimation procedure are conservative (online Technical Appendix 2, available from www.cdc.gov/EID/content/15/1/24-Techapp2.pdf).

Results

The estimated reproduction numbers decreased over time as the norovirus outbreak spread through the international scout jamboree (Figure 3). We estimated an initial reproduction number of 7.26 secondary cases per primary case (95% predictive interval 5.26–9.25); 5 days after the enhanced hygiene protocol began, the estimated reproduction number dropped below 1 (Figure 3, black diamonds). Under the hypothesis that transmission potential decreased abruptly when enhanced hygiene measures began, we estimated a reproduction number of 14.05 secondary cases per primary case (95% predictive interval 9.96–17.98) without enhanced hygiene measures and a reproduction number of 2.13 secondary cases per primary case (95% predictive interval 1.88–2.40) with enhanced hygiene measures (Figure 3, black solid line). This decrease corresponded to a relative reduction in reproduction number of 84.8% (95% predictive interval 81.2%–86.6%).

The disease attack rate varied among different camps, from 2.3% to 10.7%; overall attack rate was 7.2% (Table). For camps A and B, the estimated time course of reproduction number was initially high for the 2 index case-patients (Figure 4, black diamonds). Repeating the analysis with additional information about the host population structure and pathogen genotypes resulted in similar point estimates of the reproduction numbers (Figure 4, gray boxes) but with narrower predictive intervals. The value of the initial reproduction number in each camp followed a time course consistent with 85% reduced transmission when enhanced hygiene measures were implemented (Figure 4, black solid lines), indicating the time course of the reproduction num-

bers did not depend on the time of introduction of norovirus in the camp—because this differed between camps—but on the time the enhanced hygiene protocol began, which was identical for all camps.

Discussion

We have shown that during an outbreak of norovirus, implementation of enhanced hygiene measures coincided with an 85% reduction of norovirus transmission, from 14.05 secondary cases per primary case before enhanced hygiene measures to 2.13 secondary cases per primary case after enhanced hygiene measures. This estimate is consistent with the time course of reproduction numbers in different camps in which infection was introduced at different times. Our estimates confirm the alleged high epidemic potential of norovirus and suggest that the enhanced hygiene measures were not sufficient to reduce the reproduction number below the threshold value of 1. This estimate explains why the number of new cases per day continued to increase and why norovirus infection spread to new camps, even after implementation of enhanced hygiene measures. It is tempting to speculate that our findings could be extrapolated to other hygiene measures to explain the typical pattern in several subsequent norovirus outbreaks on cruise ships and in hotels (9,26,27).

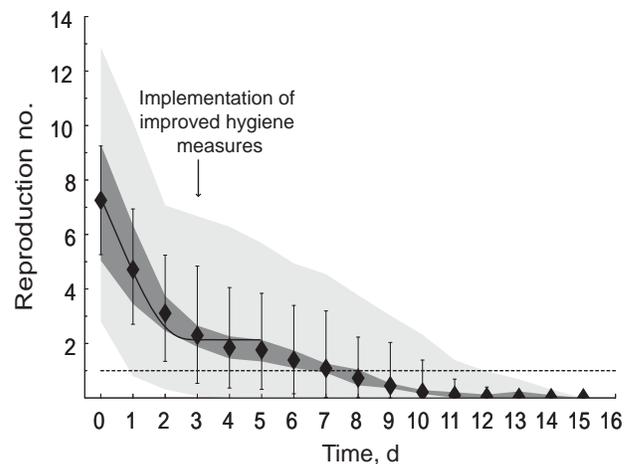


Figure 3. Time course of the reproduction number for norovirus at an international scout jamboree, starting July 26, 2004 (day 0), in the Netherlands. Black diamonds show the mean value for the reproduction number over all sampled transmission matrices; vertical lines, mean minimum and maximum values for the reproduction number over all sampled transmission matrices. The dark gray area shows the uncertainty range (0.025 and 0.975 quantiles) in the mean reproduction number; light gray area, the uncertainty range (0.025 and 0.975 quantiles) of the maximum and minimum estimates of the reproduction number. The solid black line represents the fitted time course of reproduction numbers if decrease in the mean reproduction number results from an instantaneous decline in transmission when enhanced hygiene measures began; dashed line, the threshold value of 1, below which the outbreak was controlled.

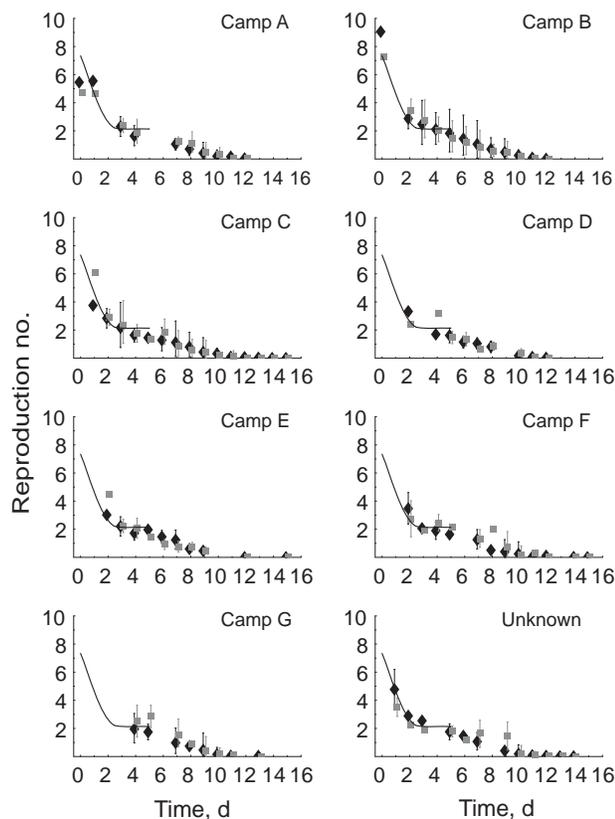


Figure 4. Time course of the reproduction number for norovirus for 7 camps of an international scout jamboree. Black diamonds show the mean value of the reproduction number without additional information about population structure and genotypes. Gray boxes show the mean value of the reproduction number when additional information about population structure and genotypes is used. The vertical lines show the mean minimum and maximum reproduction number over all sampled transmission matrices. The solid black line represents the time course of reproduction numbers if decrease in the mean reproduction number results from an instantaneous decline in transmission when enhanced hygiene measures begin. The camps are in order of the day of symptom onset of the first case-patient. Top panels indicate first introduction, bottom panels the last introduction.

The estimation procedure for the time course of the reproduction number has several limitations. It requires a frequency distribution for the generation time, which may be unknown for many diseases that are less well studied than norovirus. The procedure also requires reporting of symptom onset of case-patients over intervals on the order of the mean generation time or smaller. Here, because the mean generation time was 3.6 days, we cannot use weekly reports of time of symptom onset. The procedure also requires a large outbreak so the effects of chance events on the course of the epidemic are minimized. Small outbreaks would lead to estimates of reproduction numbers that are highly uncertain and have questionable value for making generalizations about transmission.

Our main result is that the observed decline in the reproduction number coincided with implementation of enhanced hygiene measures. This extrapolation is highly suggestive of a causal relationship, which implies that hygiene measures can effectively reduce transmission of norovirus. However, several alternatives can explain the declining reproduction number, as discussed below.

First, the decrease in reproduction number may be due to chance events. Here we explicitly estimated the reproduction numbers from times of symptom onset and the generation time distribution for norovirus infections, whereas earlier work relied on transforming epidemic curves to reproduction numbers (19,20). The tests of our explicit estimation procedure indicate that the interval estimates cover the actual values of reproduction numbers and the reduced reproduction numbers after the implementation of hygiene measures. The predictive interval for the relative reduction of 81.2%–86.6% clearly shows the change is statistically significant because it excludes the null hypothesis of a change of 0%. The tests also indicate a slight bias in the estimated values toward lower values, which suggests that the estimated 85% reduction after enhanced hygiene measures began should be treated as a conservative estimate. Therefore, the reduction in transmission is highly unlikely to be due to chance.

Second, it might be that jamboree participants differed in susceptibility, and the pool of highly susceptible persons was depleted during the first days of the outbreak. However, preexisting immunity for the genotypes involved seems highly unlikely: GI.5 and GI.4 rarely are detected in Europe, and the GII.4–2004 genotype caused a large epidemic during the winter after the jamboree (28). The number of persons infected before implementation of enhanced hygiene measures was smaller than the total number of case-patients, and the total number of case-patients was smaller than the number of jamboree participants. Depletion of susceptible persons or different susceptibility is highly unlikely to explain the sudden decrease in transmission around day 3 of the outbreak.

Third, the decline in reproduction number could be because many infections were asymptomatic and many symptomatic cases were not reported. The request to report any symptoms might not have reached all participants because of the event's large size and because participants came from many different countries. During norovirus outbreaks, asymptomatic cases occur; in almost half of the outbreaks in the Netherlands during 2002, stool samples from ≥ 1 healthy persons tested positive for norovirus (4). Volunteer and outbreak studies demonstrate that 30% of collected stool specimens of exposed, asymptomatic persons were positive for norovirus (29–31). However, both the proportion of asymptomatic infections and the reporting rate, as long as they remain constant, do not influence the value of the reproduction number because the reproduc-

tion number is estimated as the ratio of the number of secondary cases to the number of primary cases: both the proportion of asymptomatic infections and the reporting rate affect both the numerator and the denominator of this ratio, thereby canceling out in this calculation. Therefore, how the proportion of asymptomatic infections or the reporting rate would have resulted in a similar decline in reproduction number in the different camps is difficult to imagine.

Fourth, different genotypes of norovirus could have spread at different times during the outbreak. From genotyping data of 7 cases of the norovirus outbreak during the jamboree, we know that 3 different norovirus genotypes circulated during this outbreak from genogroup I and II. Recent work (32) showed first signs of a different viral load, which could indicate different transmissibility and different generation times between genogroup I and genogroup II. However, all genotyped strains were found during days 7–9 of the outbreak; although we cannot rule out the possibility that genotype replacement occurred, the most transmissible type is highly unlikely to have dominated during the first 3 days before giving way to less transmissible types.

Finally, a change of the generation time distribution during the outbreak could explain the decline in reproduction number. The method we used to estimate the time course of reproduction number depends crucially on a correct specification of the generation time distribution. Here we obtained this distribution from a study of a norovirus outbreak in child daycare centers in Sweden (25) and estimated that the generation time distribution peaked at 2.6 days (Figure 2). This estimation agrees with results from volunteer studies in which adults showed a peak in virus shedding at 1–3 days postchallenge (31). Further, this peak agrees with the time between exposure and symptom onset of 2 days in primary-school children during a norovirus outbreak after a vomiting event (6), whereas 80% of case-patients reported vomiting during the scout jamboree. Overall, the most plausible explanation for the decrease in reproduction number is implementation of enhanced hygiene measures.

We have quantitatively estimated the effectiveness of enhanced hygiene measures in containing an outbreak of norovirus. Because the reproduction number did not fall below the threshold value of 1, implementation of the hygiene measures was not sufficient to effectively break the chain of person-to-person transmission of norovirus during this outbreak. To contain an outbreak of norovirus, more rigorous interventions are required. These might range from better compliance with hygiene protocols to strict isolation of case-patients and quarantine of their contacts. We recommend quantifying the effectiveness of interventions against norovirus to provide the necessary evidence to justify use of existing hygiene protocols during outbreaks and to direct

the development of better intervention measures. Although such quantifying would require analysis of more norovirus outbreaks with different sets of intervention measures, it would enable identification of the best possible intervention strategies to control the spread of one of the most common pathogens of humans.

Acknowledgments

We thank Harry Vennema and Bas van der Veer for performing the norovirus diagnostic testing and Nicola Low for commenting on the final version.

Ms Heijne is an infectious disease epidemiologist, formerly at the National Institute for Public Health and the Environment in the Netherlands and now at the Institute of Social and Preventive Medicine, University of Bern in Switzerland. Her work focuses on analyzing time series of infectious diseases.

References

1. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. *Emerg Infect Dis.* 1999;5:607–25.
2. Estes MK, Prasad BV, Atmar RL. Noroviruses everywhere: has something changed? *Curr Opin Infect Dis.* 2006;19:467–74. DOI: 10.1097/01.qco.0000244053.69253.3d
3. Kroneman A, Vennema H, Harris J, Reuter G, von Bonsdorff CH, Hedlund KO et al. Increase in norovirus activity reported in Europe. *Euro Surveill* 2006;11(50):3093 [cited 17 Nov 2008]. Available from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3093>
4. van Duynhoven YT, de Jager CM, Kortbeek LM, Vennema H, Koopmans MP, van Leusden F, et al. A one-year intensified study of outbreaks of gastroenteritis in the Netherlands. *Epidemiol Infect.* 2005;133:9–21. DOI: 10.1017/S0950268804002936
5. Duizer E, Koopmans M. Tracking foodborne viruses: lessons from noroviruses. In: Motarjemi Y, Adams M, editors. *Emerging foodborne pathogens*. Boca Raton (FL): CRC Press, 2006. p. 77–110.
6. Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI, et al. An outbreak of viral gastroenteritis following environmental contamination at a concert hall. *Epidemiol Infect.* 2002;129:355–60. DOI: 10.1017/S0950268802007446
7. Marks PJ, Vipond IB, Carlisle D, Deakin D, Fey RE, Caul EO. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. *Epidemiol Infect.* 2000;124:481–7. DOI: 10.1017/S0950268899003805
8. Barker J, Vipond IB, Bloomfield SF. Effects of cleaning and disinfection in reducing the spread of norovirus contamination via environmental surfaces. *J Hosp Infect.* 2004;58:42–9. DOI: 10.1016/j.jhin.2004.04.021
9. Isakbaeva ET, Widdowson MA, Beard RS, Bulens SN, Mullins J, Monroe SS, et al. Norovirus transmission on cruise ship. *Emerg Infect Dis.* 2005;11:154–8.
10. Love SS, Jiang X, Barrett E, Farkas T, Kelly S. A large hotel outbreak of Norwalk-like virus gastroenteritis among three groups of guests and hotel employees in Virginia. *Epidemiol Infect.* 2002;129:127–32. DOI: 10.1017/S0950268802007161
11. Rockx B, De Wit M, Vennema H, Vinje J, De Bruin E, Van Duynhoven Y, et al. Natural history of human calicivirus infection: a prospective cohort study. *Clin Infect Dis.* 2002;35:246–53. DOI: 10.1086/341408

12. LoBue AD, Lindesmith L, Yount B, Harrington PR, Thompson JM, Johnston RE, et al. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. *Vaccine*. 2006;24:5220–34. DOI: 10.1016/j.vaccine.2006.03.080
13. Calderon-Margalit R, Sheffer R, Halperin T, Orr N, Cohen D, Shohat T. A large-scale gastroenteritis outbreak associated with norovirus in nursing homes. *Epidemiol Infect*. 2005;133:35–40. DOI: 10.1017/S0950268804003115
14. Cheng FW, Leung TF, Lai RW, Chan PK, Hon EK, Ng PC. Rapid control of norovirus gastroenteritis outbreak in an acute paediatric ward. *Acta Paediatr*. 2006;95:581–6. DOI: 10.1080/08035250500449874
15. Navarro G, Sala RM, Segura F, Arias C, Anton E, Varela P, et al. An outbreak of norovirus infection in a long-term-care unit in Spain. *Infect Control Hosp Epidemiol*. 2005;26:259–62. DOI: 10.1086/502536
16. Schmid D, Lederer I, Pichler AM, Berghold C, Schreier E, Allerberger F. An outbreak of norovirus infection affecting an Austrian nursing home and a hospital. *Wien Klin Wochenschr*. 2005;117:802–8. DOI: 10.1007/s00508-005-0473-1
17. Cheesbrough JS, Green J, Gallimore CI, Wright PA, Brown DW. Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiol Infect*. 2000;125:93–8. DOI: 10.1017/S095026889900432X
18. Verhoef L, Depoortere E, Boxman I, Duizer E, Van Duynhoven Y, Harris J, et al. Emergence of new norovirus variants on spring cruise ships and prediction of winter epidemics. *Emerg Infect Dis*. 2008;14:238–43. DOI: 10.3201/eid1402.061567
19. Wallinga J, Teunis P. Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. *Am J Epidemiol*. 2004;160:509–16. DOI: 10.1093/aje/kwh255
20. Cauchemez S, Boelle PY, Donnelly CA, Ferguson NM, Thomas G, Leung GM, et al. Real-time estimates in early detection of SARS. *Emerg Infect Dis*. 2006;12:110–3.
21. Duizer E, Timen A, Morroy G, de Roda Husman AM. Norovirus outbreak at an international scout jamboree in the Netherlands, July–August 2004: international alert. *Euro Surveill*. 2004;8(33):2523 [cited 17 Nov 2008]. Available from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2523>
22. Morroy G, Wijkman C. A norovirus outbreak at an international scouting jamboree in the Netherlands [in Dutch]. *Infectieziekten bulletin*. 2005;16:57–9 [cited 17 Nov 2008]. Available from http://www.rivm.nl/infectieziektenbulletin/bul1602/veld_jamboree.html
23. Vennema H, De Bruin E, Koopmans M. Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. *J Clin Virol*. 2002;25:233–5. DOI: 10.1016/S1386-6532(02)00126-9
24. Fine PE. The interval between successive cases of an infectious disease. *Am J Epidemiol*. 2003;158:1039–47. DOI: 10.1093/aje/kwg251
25. Götz H, Ekdahl K, Lindback J, de Jong B, Hedlund KO, Giesecke J. Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden. *Clin Infect Dis*. 2001;33:622–8. DOI: 10.1086/322608
26. Enserink M. Infectious diseases: gastrointestinal virus strikes European cruise ships. *Science*. 2006;313:747. DOI: 10.1126/science.313.5788.747a
27. Koopmans M, Harris J, Verhoef L, Depoortere E, Takkinen J, Coulombier D. European investigation into recent norovirus outbreaks on cruise ships: update. *Euro Surveill*. 2006;11(27):2997 [cited 17 Nov 2008]. Available from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2997>
28. Siebenga JJ, Vennema H, Renckens B, De Bruin E, van der Veer B, Siezen RJ, et al. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. *J Virol*. 2007;81:9932–41. DOI: 10.1128/JVI.00674-07
29. Gallimore CI, Cubitt D, du Plessis N, Gray JJ. Asymptomatic and symptomatic excretion of noroviruses during a hospital outbreak of gastroenteritis. *J Clin Microbiol*. 2004;42:2271–4. DOI: 10.1128/JCM.42.5.2271-2274.2004
30. Garcia C, DuPont HL, Long KZ, Santos JI, Ko G. Asymptomatic norovirus infection in Mexican children. *J Clin Microbiol*. 2006;44:2997–3000. DOI: 10.1128/JCM.00065-06
31. Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk virus infection of volunteers: new insights based on improved assays. *J Infect Dis*. 1994;170:34–43.
32. Chan MC, Sung JJ, Lam RK, Chan PK, Lee NL, Lai RW, et al. Fecal viral load and norovirus-associated gastroenteritis. *Emerg Infect Dis*. 2006;12:1278–80.

Address for correspondence: Janneke C.M. Heijne, Institute of Social and Preventive Medicine, University of Bern, Finkenhubelweg 11, 3012 Bern, Switzerland; email: jheijne@ispm.unibe.ch

EMERGING INFECTIOUS DISEASES *online*

www.cdc.gov/eid

To receive tables of contents of new issues send an email to listserv@cdc.gov with `subscribe eid-toc` in the body of your message.

Enhanced Hygiene Measures and Norovirus Transmission during an Outbreak

Technical Appendix 1: Methods

Estimation of the Generation Time Distribution

Time interval data from large norovirus outbreaks in Sweden in 1999 (norovirus genogroup II) in childcare centers (I) were used to estimate the generation time distribution by the maximum likelihood method (Figure 2 in main text). The input data consist of a vector of the observed time intervals \mathbf{s} , with s_1, s_2, \dots, s_n denoting the times between symptom onset in persons who attended a childcare center and the times of symptom onset in household members of the infected persons.

We assume that the generation time distribution follows a gamma distribution with a shape parameter α and a scale parameter β . The log-likelihood for the observed time intervals \mathbf{s} , given the parameters for the generation time, is

$$l(\alpha, \beta | \mathbf{s}) = \log \left(\prod_{i=1}^n \text{Gamma}(s_i | \alpha, \beta) \right).$$

The estimated maximum likelihood estimates are $\alpha = 3.35$ and $\beta = 1.09$, resulting in a peak generation time of 2.6, and a mean generation time of 3.6 days. Other positively skewed unimodal distributions such as the Weibull distributions did not produce a significantly better fit. As the generation time distribution might also be a realization of a mixture of several components, we fitted the data with a mixture of 2 or 3 gamma distributed components. This did not give a significantly better fit than a 1-component model (Technical Appendix 1 Table).

Technical Appendix 1 Table. Summary of a fit of gamma distribution with 1, 2, or 3, components, respectively, to the serial interval data

No. components	log likelihood	Deviance	Degrees of freedom	p value
1	345.176			
2	338.290	6.886	3	0.076
3	332.609	12.567	6	0.051

Estimation of the Effective Reproduction Number, R

Definition of Transmission Matrix

Let $\mathbf{t} = (t_1, \dots, t_n)$ be the vector of observed times of symptom onset of observed cases $\{1, \dots, n\}$. We assume that the elements of t are ordered such that $t_i \leq t_j$ for all $i < j$. For subsets $\{i_k, \dots, i_{k+j}\} \subseteq \{1, \dots, n\}$ with $t_{i_k} = t_{i_{k+1}} = \dots = t_{i_{k+j}}$ all permutations of observations within this subset are equivalent. We chose 1 possible ordering arbitrarily. We now define a transmission matrix $V = (v_{i,j})$, whose elements represent the probability that the person with time of symptom onset t_i was infected by the person with time of symptom onset t_j , thus $v_{i,j} \in [0,1]$. Assuming that every case $i \geq 2$ was infected by another case in the set of observed cases, we get:

$$\sum_{j=1}^n v_{i,j} = 1$$

for all $i \geq 2$. For $i=1$, the index case, we assume that:

$$\sum_{j=1}^n v_{i,j} = 0.$$

Furthermore, we assume that $v_{i,j} = 0$ for all $j \leq i$. This assumption means that the ordering of times of infection is equivalent to the ordering of observed times of symptom onset, and more specifically, that persons cannot have infected themselves and cannot have infected persons with earlier time of symptom onset than their own. The matrix V is a lower triangular matrix and therefore does not contain cycles.

Translation of Transmission Matrix to Reproduction Number Estimates

To translate the transmission matrix V to reproduction number estimates, any transmission matrix V may represent many different transmission trees. A transmission tree consists of nodes representing all cases of the outbreak and direct edges between nodes representing transmission of infection between the cases.

Let a transmission tree be represented by a binary matrix $U = (u_{i,j})$ of infectious contacts with $u_{i,j} = 1$ if case i is infected by case j and $u_{i,j} = 0$ if case i is not infected by case j . The row vector in matrix U can be seen as a draw from a multinomial distribution of order 1 (each case-patient received his or her infection from exactly 1 other case-patient) and a probability equal to

a row from matrix V , producing a vector u_i of 0s and only 1 element equal to 1: $u_i = \text{multinomial}(1, v_i)$.

With a transmission tree, it is possible to simulate an epidemic curve. For any pair of cases i, j of which $u_{i,j} = 1$ draw a generation time τ_i from the generation time distribution $g(\tau | \theta)$, with θ being the parameters of the generation time distribution. With the generation time τ_i , the time of infection of case i can be determined: $t_i = t_j + \tau_i$. If the time of infection is known from the index case, all times of infections in all other cases can be determined, which results in an epidemic curve.

The expected number of secondary cases produced by case j in these possible outbreaks based on transmission matrix V is:

$$E_v(R_j) = \sum_i E(u_{i,j}) = \sum_i v_{i,j}$$

To translate this to an estimate of R for each day in the outbreak t , the mean R_j of all cases with the same date of symptom onset is calculated, for all dates with observations:

$$E_v(R(t)) = \frac{1}{q} \sum_{j=m}^{m+q} E_v(R_j)$$

where m represents the label of the first case with symptom onset on day t , and q the total number of cases on day t .

Likelihood Function

The likelihood that an observed time interval $t_i - t_j$ represents a transmission event is determined as a product of the probability that i was infected by j and the probability that the time interval of symptom onset is $t_i - t_j$. That is,

$$L_{i,j}(\theta, v_{i,j} | t_i - t_j) = g(t_i - t_j | \theta) \cdot v_{i,j}$$

The likelihood of any case-patient j transmitting infection to case-patient i , becomes:

$$L_i(\theta, v_i | t_i - t_j) = \sum_{j=1}^n (g(t_i - t_j | \theta) \cdot v_{i,j})$$

Combining for all observed cases, the likelihood of a transmission matrix \mathbf{V} becomes:

$$L(\mathbf{V}|\mathbf{t}) = \prod_{i=2}^n \left(\sum_{j=1}^n \left(g(t_i - t_j | \theta) \cdot v_{i,j} \right) \right)$$

for a given value of θ , and omitting the index case ($i = 1$) from the multiplication. Given the parameters for the generation time distribution θ , and all dates of symptom onset t , the parameters $v_{i,j}$ can be estimated. To estimate $v_{i,j}$, the above likelihood function was evaluated in an adaptive rejection algorithm (Metropolis Hastings sampler) obtaining sets of \mathbf{V} matrices with relative frequencies proportional to their likelihood (2–4).

To be reasonably certain of convergence and sufficient mixing, we have run 4 independent chains of 40,000 iterations and 3 independent chains with additional information about population structure and pathogen genotype and compared resulting estimates of reproduction numbers.

Adding additional information is possible by setting implausible transmission probabilities in the transmission matrix \mathbf{V} to 0. This may be considered a very strong prior assumption, but we have seen (Figure 4 in main text) that the resulting reproduction numbers are not strongly influenced by this radical assumption. In a true Bayesian approach, we might have applied different weights to pairs of cases within and between camps by multiplying a matrix containing these weights with the transmission matrix \mathbf{V} .

As described above, case-patients with a date of symptom onset on the same day are given an arbitrary order of infection within that day. Sampled transmission matrices represent all possible (noncyclic) patterns among cases, given the arbitrary order. Now any other possible pattern can be found by permutation of indexes among cases with the same date of symptom onset. Because these all have the same contribution to the likelihood such permutations do not change the likelihood: all permutations are equally likely. Such permutations also have the same reproduction numbers, only for different cases (indices). If we average over all such permutations with identical contributions, the resulting reproduction numbers do not change.

Expected Time Course of Reproduction Number

The expected time course of the reproduction number $R(t)$ is given by the following equation:

$$R(t) = (G(t_h - t) + (1 - G(t_h - t))\rho)R_u$$

Here, t_h is the day of implementation of enhanced hygiene measures, G is the cumulative probability function of the generation time distribution, ρ is the relative reduction of the reproduction number due to implementation of hygiene measures and R_u is the effective reproduction number without enhanced hygiene measures.

References

1. Götz H, Ekdahl K, Lindback J, de Jong B, Hedlund KO, Giesecke J. Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden. *Clin Infect Dis*. 2001;33:622–8. [PubMed DOI: 10.1086/322608](#)
2. Gilks WR, Richardson S, Spiegelhalter DJ. *Markov chain Monte Carlo in practice*. London: Chapman & Hall/CRC Press; 1995.
3. O'Neill PD. A tutorial introduction to Bayesian inference for stochastic epidemic models using Markov chain Monte Carlo methods. *Math Biosci*. 2002;180:103–14. [PubMed DOI: 10.1016/S0025-5564\(02\)00109-8](#)
4. O'Neill PD, Marks PJ. Bayesian model choice and infection route modelling in an outbreak of Norovirus. *Stat Med*. 2005;24:2011–24. [PubMed DOI: 10.1002/sim.2090](#)

Enhanced Hygiene Measures and Norovirus Transmission during an Outbreak

Technical Appendix 2: Testing the Estimation Procedures with Simulated Outbreaks

Simulation Study 1: Testing the Estimated Time Course of Reproduction Numbers

We used an individual-based stochastic model to simulate 50 epidemic curves. For any case with symptom onset at day t , the number of secondary cases is sampled from a geometric distribution with a mean equal to $R(t)$ as estimated from the outbreak data (black diamonds in Figure 3 in the main article). For each of these secondary cases, the generation time is sampled from a gamma distribution with parameters $\alpha = 3.35$ and $\beta = 1.09$ with a mean of 3.6 days, as estimated from the observed generation times (Technical Appendix 1, available from www.cdc.gov/EID/content/15/1/24-Techapp1.pdf). Each simulated outbreak started with 3 initial cases at day 0.

We used the same estimation procedure as described in Technical Appendix 1 to estimate the time course of the mean value of the reproduction numbers $R(t)$. We used fewer samples of the transmission matrix than for the actual estimates in the main text.

Simulation Study 2: Testing Estimation of Impact of Intervention Measures

We simulated again 50 epidemic curves with an individual-based stochastic model. For each case, the number of secondary cases is sampled from a geometric distribution with mean corresponding to the estimated mean reproduction number without enhanced hygiene measures R_u of 14.05, and an instantaneous decrease in reproduction number ρ of 85% when enhanced hygiene measures are implemented (black solid line in Figure 3 in main text).

Evaluation of Simulation Studies

The test results show that the point estimates of reproduction numbers in simulated outbreaks closely follows the actual value of reproduction numbers, but are biased toward lower values than the actual ones (Technical Appendix 2 Table 1). The ranges of estimated reproduction numbers cover the actual values. The test results also show a downward bias in the estimates of the reproduction number without enhanced hygiene measures R_u and the relative reduction in reproduction numbers ρ (Technical Appendix 2 Table 2). The downward bias can be attributed to the so-called attenuation bias of the least squares regression that was used to estimate the parameters R_u and ρ . Attenuation bias is caused by random noise in the explanatory variable, which induces a bias in the estimated regression coefficient toward 0. Here, random noise is introduced in the time of symptom onset by the variability in generation times, and this causes a bias of the parameter ρ toward 0.

Technical Appendix 2 Table 1. Test results for the estimation procedure of reproduction numbers*

Parameter	Actual value	Estimated value, mean (range)
$R(0)$	7.3	5.1(1.7–8.3)
$R(1)$	4.7	3.4(1.6–4.8)
$R(2)$	3.1	2.7(0.6–3.6)
$R(3)$	2.3	2.1(0.8–3.0)
$R(4)$	1.9	1.8(0.6–2.3)
$R(5)$	1.8	1.4(0.2–1.8)
$R(6)$	1.4	1.1(0.04–1.5)
$R(7)$	1.1	0.8(0.3–1.2)

*Range, minimum and maximum value of the estimated mean reproduction numbers in 50 simulations; $R(t)$, mean reproduction number of cases with symptom onset on day t in 50 simulations.

Technical Appendix 2 Table 2. Test results for the estimation procedure of the impact of enhanced hygiene measures*

Parameter	Actual value	Estimated value, mean (range)
R_u	14.1	9.5(3.7–15.2)
$(1-\rho)R_u$	2.1	2.1(1.2–2.6)
ρ	0.85	0.77(0.59–0.86)

*Range, minimum and maximum value of the estimated mean parameters in 50 simulations; R_u , mean reproduction number without enhanced hygiene measures; $(1-\rho)R_u$, mean reproduction number with enhanced hygiene measures; ρ , relative reduction in reproduction number when enhanced hygiene measures began.