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Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces

J. Barker^{a,*}, I.B. Vipond^b, S.F. Bloomfield^c

^aDepartment of Pharmaceutical and Biological Sciences, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK

^bHealth Protection Agency—South West, Regional Virus Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL, UK

^cLondon School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

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Summary A reverse transcriptase polymerase chain reaction assay was used to study the transfer of Norovirus (NV) from contaminated faecal material via fingers and cloths to other hand-contact surfaces. The results showed that, where fingers come into contact with virus-contaminated material, NV is consistently transferred via the fingers to melamine surfaces and from there to other typical hand-contact surfaces, such as taps, door handles and telephone receivers. It was found that contaminated fingers could sequentially transfer virus to up to seven clean surfaces. The effectiveness of detergent- and disinfectant-based cleaning regimes typical of those that might be used to decontaminate faecally contaminated surfaces and reduce spread of NV was also compared. It was found that detergent-based cleaning with a cloth to produce a visibly clean surface consistently failed to eliminate NV contamination. Where there was faecal soiling, although a combined hypochlorite/detergent formulation at 5000 ppm of available chlorine produced a significant risk reduction, NV contamination could still be detected on up to 28% of surfaces. In order consistently to achieve good hygiene, it was necessary to wipe the surface clean using a cloth soaked in detergent before applying the combined hypochlorite/detergent. When detergent cleaning alone or combined hypochlorite/detergent treatment failed to eliminate NV contamination from the surface and the cleaning cloth was then used to wipe another surface, the virus was transferred to that surface and to the hands of the person handling the cloth. In contrast, were surfaces where contaminated with NV-infected faecal suspension diluted to 1 in 10 and 1 in 80, intended to simulate surfaces that have become contaminated after secondary transfer,

*Corresponding author.

E-mail address: j.e.barker@aston.ac.uk

treatment with a combined bleach/detergent formulation, without prior cleaning, was sufficient to decontaminate surfaces and prevent transfer.
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Introduction

Noroviruses (NVs) are a major cause of gastroenteritis, which spreads rapidly in premises such as hospitals, hotels, day-care centres, residential and domestic homes.¹⁻⁵ NV hospital outbreaks are common, and frequently result in ward closures because of the need to prevent further spread and to facilitate environmental cleaning. The scale of the problem in the community was highlighted by a recent study of rates of infectious intestinal disease in the UK. Wheeler *et al.*⁶ estimated that for every one case of NV reported to national surveillance, a further 1562 cases occur in the community. Based on the number of reported cases, Evans *et al.*⁷ estimated the total number of cases in the community to be around 3 million per year.

The likelihood of airborne transmission of NV was demonstrated in an outbreak at a restaurant where no food source was implicated, but an analysis of the attack rate showed an inverse correlation with the distance from a person who had vomited.³ Microbiological data show that projectile vomiting associated with NV infection may distribute up to 3×10^7 virus particles as an aerosol,⁸ whilst environmental sampling during outbreaks of vomiting and diarrhoea confirm that widespread dissemination of NV occurs on hand contact and other surfaces.^{2,9} From such data and from estimates which suggest that the infective dose for NV may be as low as 10-100 particles, Caul⁸ concluded that, in addition to possible aerosol inhalation, hands and surfaces also play an important part in facilitating transfer of NV infection, either by direct faecal-oral transfer or by transfer to foods that are eaten without further cooking.

The potential for transmission of NV via environmental surfaces is also supported by epidemiological studies, the most compelling evidence coming from recurrent outbreaks of NV infection in successive cohorts of guests in hotels and on cruise ships.⁹⁻¹² From the patterns of infection, it is concluded that whereas aerosols are probably the main route of dissemination within a cohort of guests, contaminated fomites are the most likely factor responsible for sustaining a succession of outbreaks. Other data comes from an investigation of an NV outbreak at a wedding reception, which showed that the likely route of transmission was

from a kitchen assistant who had vomited in a sink that was subsequently used for preparing vegetables eaten by the wedding guests.¹³ Analysis of risk exposure during an outbreak of NV gastroenteritis in an elderly care unit showed that areas where patients had vomited were the most significant factor for the spread of NV to staff.¹ Further evidence of environmental transfer comes from a report of two carpet fitters who became ill after removing a carpet from a hospital ward 13 days after the last case in an NV outbreak.¹⁴ The role of contaminated soft furnishings in transmission and the difficulties these pose for decontamination is highlighted by the outbreak that occurred among school children who attended a theatre in which a vomiting incident had occurred on a previous day.¹⁵ Guidelines have been issued for the control of NV outbreaks in hospitals that stress the importance of preventing staff and patient movements to other areas, thorough handwashing and effective environmental decontamination.¹⁶

Since NV is uncultivable in the laboratory, little is known about the length of time it remains infectious in the environment or the effectiveness of disinfection procedures used to inactivate the virus. The development of a broadly reactive reverse transcriptase polymerase chain reaction (RT-PCR) has facilitated the detection of the virus in both clinical and environmental samples.¹⁷ In this investigation, we have used an RT-PCR assay to study the transfer of NV from contaminated faecal material via fingers and surfaces, and compared the effectiveness of detergent-based cleaning alone with hypochlorite disinfection for eliminating NV from faecally contaminated surfaces.

Materials and methods

NV-contaminated faecal sample

A homogenized clinical faecal sample (obtained from Bristol Public Health Laboratory) that was positive for NV genogroup II when assayed by RT-PCR was used throughout. The number of virus particles per gram of sample was not known, but it was found that positive amplicons could be obtained by RT-PCR up to a dilution of 1:2000 of the sample (data not shown). A faecal sample

negative for NV when assayed by RT-PCR was used as a negative control.

Transfer of NV via fingers and surfaces

To contaminate fingers, 150 μ L of the faecal sample, diluted 1:5 in phosphate-buffered saline (PBS, Oxide, UK), was absorbed on to toilet paper in a Petri dish and the fingertips of the experimenter were pressed on to the contaminated tissue for 10 s. The fingers were allowed to dry for 15 s at room temperature before sampling for the presence of NV. Preliminary studies involving 10 replicate tests (data not shown) showed that NV could be consistently recovered from fingertips after contact with faecally contaminated toilet tissue. A further 10 replicate tests showed that if virus-contaminated hands were washed thoroughly with liquid soap and water for 1 min using the recommended 'five-step' handwashing procedure,¹⁸ followed by rinsing for 20 s and drying using disposable paper towels, no virus was then detectable.

To study transfer to surfaces, the fingers were contaminated and allowed to dry for 15 s, as described above. The contaminated fingertips were then pressed on to clean melamine surfaces for 10 s. The surfaces were left at room temperature for 15 min before sampling for the presence of NV.

To determine secondary transfer of NV, melamine surfaces were contaminated by contact with fingers as described above. After allowing the contaminated melamine surface to dry at room temperature for 15 min, it was touched by clean dry fingers which were then used to touch a telephone receiver, a tap handle and a door handle. The secondary surfaces were left at room temperature for 15 min before sampling for the presence of NV.

At the end of each experiment, the melamine and other surfaces used in this study were disinfected with a hypochlorite disinfectant/cleaner (HDC) containing 5000 ppm of available chlorine and 4% (w/v) of an anionic surfactant (supplied by Lever Brothers, Port Sunlight, UK) for a contact time of 1 h. The surfaces were then rinsed in running water for 2 min and dried. Preliminary experiments revealed that these procedures were effective in eliminating NV.

Cleaning and disinfection studies

Six melamine surfaces were contaminated directly with 10 μ L faecal sample (diluted 1:5, 1:10 or 1:80 in PBS), which was spread over a demarcated area of 15 \times 15 mm and allowed to dry at room tem-

perature for 15 min. One surface was used as an untreated control and for the remaining surfaces, a comparison was made of five different cleaning and disinfection protocols:

Test a: cleaning with a cloth soaked in detergent solution for 10 s;

Test b: cleaning with a cloth soaked in detergent solution for 10 s. The cloth was rinsed in detergent solution wrung out and used to rewipe the surface for 10 s;

Test c: HDC was applied to the surface for 1 min. A cloth soaked in detergent solution was then used to wipe the disinfected surface for 10 s;

Test d: as for test c except HDC was applied for 5 min;

Test e: gross faecal matter was removed from the surface by an initial wipe with a cloth soaked in detergent solution for 10 s, and the surface was then disinfected with HDC as in test c.

Immediately after cleaning or cleaning and disinfection, the melamine surfaces were sampled and assayed by RT-PCR. The wiping cloth was then used to wipe a separate clean surface.

The melamine surface and the fingers of the hand that had handled the wiping cloth were sampled and assayed by RT-PCR.

For cleaning, a solution of anionic detergent diluted to 0.04% in tap water was applied to the faecally contaminated melamine surface with a 'J' cloth (15 \times 15 mm). It was found that after wiping for 10 s, no visible faecal soiling remained. Disinfection was carried out by applying 40 μ L of the HDC, containing 5000 ppm available chlorine, to the faecally contaminated surface to ensure that the soiled area was completely covered.

Sampling and detection of virus by RT-PCR

Fingers and other surfaces were sampled using a cotton swab moistened in PBS and sodium thiosulphate 0.2% (w/v) to neutralize residual disinfectant.^{19,20} Preliminary tests had revealed that this level of sodium thiosulphate was capable of neutralizing the residual effects of sodium hypochlorite up to concentrations of 5000 ppm (data not shown). The swab was placed in 300 μ L of diluent and homogenized by vortex mixing for 30 s. Samples prepared in the diluent with and without added sodium thiosulphate showed that it did not interfere with the RT-PCR.

The QIAamp viral RNA mini kit (Qiagen, UK) was used for extraction and purification of viral RNA from the diluted homogenized faecal sample or

swab sample as described by Taku *et al.*²¹ The extracted RNA was either used directly for RT-PCR or stored at -70°C . The cDNA was prepared by reverse transcription (RT) by adding 36 μL RNA extract to 14 μL of the following mixture. MMLV reverse transcriptase at 200 units/ μL (Life Technologies, UK), 5 \times 1st stand buffer (supplied with reverse transcriptase), and 10 mM each of deoxynucleoside triphosphates and random hexamers at 500 $\mu\text{g}/\text{mL}$ (Pharmacia, UK). RT was carried out at 37°C for 1 h. The cDNA was used immediately or stored at -70°C .

Inosine-containing primers (50 $\mu\text{g}/\text{mL}$) were used for PCR with the following nucleotide sequences: G7 (group 2) 5' GAI GGI CTI CCA TCW GGI TTY CC3' and Y5 (universal) 5' ACI ATY TCR TCA TCI CCR TAR AA3'. RT-PCR was performed by adding 2 μL cDNA to 18 μL reaction mixture containing: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X100, 0.2 mM deoxynucleoside triphosphates, 20 pmol of each primer (G7 and Y5), and 0.5 units of Taq polymerase. Initial denaturation was carried out at 94°C for 2 min 40 s (step 1 cycle 1) followed by denaturation at 94°C for 20 s, annealing at 50°C for 20 s and extension at 72°C for 10 s, for 40 cycles of amplification. PCR amplicons were analysed by electrophoresis of 20 μL reaction mixture in agarose gels. The presence of a 155 bp PCR product indicated a NV group 2 positive result.

Results

Contamination of fingers and transfer of NV to surfaces

In a series of four replicate experiments, faecally contaminated fingertips were used sequentially to touch a series of eight clean melamine surfaces, without recontaminating the fingers for each new surface touched (Figure 1). For the first four surfaces in the series, all four replicates were positive for NV. For surfaces five and six, three out of four replicates were positive, for surface seven, one out of four was positive, and for surface eight, all four replicates were negative. In a further series of experiments, melamine surfaces were contaminated by contact with faecally contaminated fingers and secondary transfer of NV via hands to other surfaces was determined. By touching the NV-contaminated surfaces with clean fingers, it was found that NV was transferred from the primary surface to four out of 10 door handles, five out of 10 telephone receivers and three out of 10 taps.

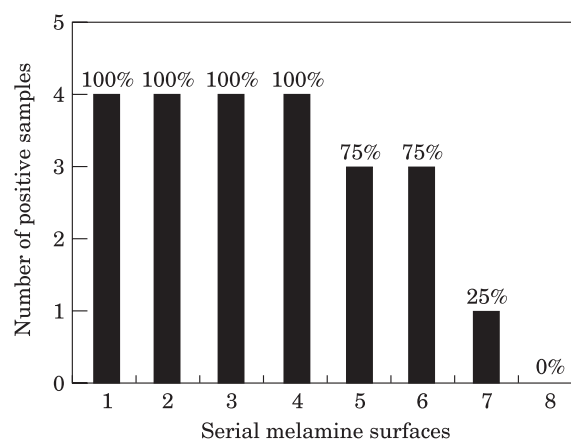


Figure 1 Sequential finger transfer of NV to clean melamine surfaces after initial contamination of fingers with faecally contaminated toilet tissue (four replicate tests).

Surface decontamination

Cleaning of melamine surfaces contaminated with the homogenized faecal sample with detergent solution did not eliminate NV (Figure 2). This was the case even where the cloth was washed in fresh detergent solution, wrung out and the wiping process was repeated. In all 14 replicate tests, residual NV was detected on the melamine surfaces. It was also found that where the cloth was handled and used to wipe a second clean melamine surface, the virus could be recovered from not only the second surface but also from the fingers in all cases.

In a proportion of replicate tests, cleaning and disinfecting the contaminated surfaces with HDC containing 5000 ppm available chlorine was effective in eliminating NV from the surface and preventing cross-contamination. After 1 min and 5 min contact time with HDC, the number of NV-positive surfaces was reduced to 21% or 28%, respectively. Where the HDC-treated surface tested negative for NV, the virus was also absent from the second surface and the fingers. On the four occasions where disinfection was insufficient to eliminate NV, transfer of NV to the second clean surface was recorded. On three of these occasions, the cloth also spread the virus to the fingers. In contrast, after cleaning the surfaces with detergent solution, followed by HDC treatment at 5000 ppm for 1 min, no NV was detected on the surfaces.

Further tests were carried out to determine the effectiveness of cleaning and disinfection procedures on surfaces contaminated with dilute faecal suspensions. Melamine surfaces were contaminated with NV-contaminated faecal

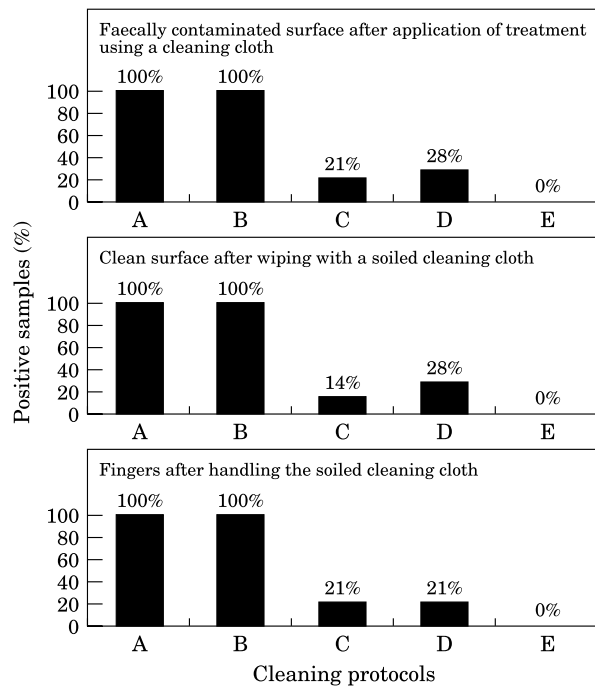


Figure 2 Comparison of the effectiveness of detergent-based and hypochlorite/detergent-based cleaning procedures in elimination of faecal contamination from surfaces and prevention of transfer to clean surfaces and hands. Surfaces were contaminated with a 1:5 dilution of a faecal suspension infected with NV. (A) Surface cleaned with detergent and water; (B) surface cleaned with detergent and water, cloth rinsed and used to rewipe surface; (C) surface treated with hypochlorite 5000 ppm (1 min); (D) surface treated with hypochlorite 5000 ppm (5 min); (E) surface cleaned with detergent and water and treated with hypochlorite 5000 ppm (1 min; 14 replicate tests).

suspensions diluted to either 1 in 10 or 1 in 80 in PBS. The results (Figure 3) show that detergent-based cleaning, even with a second wipe step, failed to decontaminate surfaces, and in all but one case (transfer to the fingers from the most lightly contaminated surface), the virus was spread to the clean surface and the fingers via the wiping cloth. In contrast, where the surface was treated with HDC containing 5000 ppm available chlorine applied for 1 min, for all except one of the surfaces, which had the heavier soiling, no NV could be recovered and no cross-contamination was detected.

Discussion

This investigation established a model to study transmission of NV from an infectious source via fingers, cloths and contact surfaces. RT-PCR was used to confirm transfer of NV from surface to surface. In the absence of a tissue-culture method

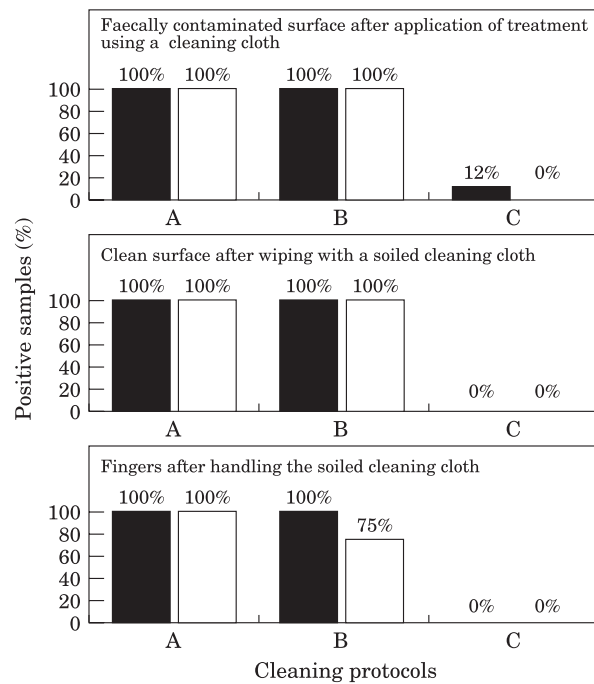


Figure 3 Comparison of the effectiveness of detergent-based and hypochlorite/detergent-based cleaning procedures in elimination of faecal contamination from surfaces and prevention of transfer to clean surfaces and hands. Surfaces were contaminated with a 1:10 (■) and 1:80 (□) dilution of a faecal suspension infected with NV. (A) Surface cleaned with detergent and water; (B) surface cleaned with detergent and water, cloth rinsed and used to rewipe surface; (C) surface treated with hypochlorite 5000 ppm (1 min; eight replicate tests).

for NV, the correlation between viral RNA detection and numbers of virus particles and/or their infectivity is impossible to determine. Thus, although it can be argued that the absence of detectable RNA is likely to indicate the absence of infectious viral particles, the opposite does not necessarily apply. Nevertheless, the results of the study indicate that where fingers come into contact with virus-contaminated toilet tissue, NV is consistently transferred via the fingers to a melamine surface and from there to other typical hand-contact surfaces such as taps, door handles and telephone receivers. It was found that contaminated fingers could transfer virus to up to seven clean surfaces touched sequentially. These results are in agreement with those of Rheinbaben *et al.*²² who studied virus transmission in a household and found that door handles and hands were efficient vectors for bacteriophage transfer. At least 14 persons could be contaminated one after another by touching a contaminated door handle. Jiang *et al.*²³ similarly showed that where a marker DNA virus was introduced into childcare facilities through treated

toy balls, the marker was widely disseminated to environmental surfaces. In a study of homes where there was an infant recently vaccinated for polio (during which time shedding occurs in faeces), Curtis *et al.*²⁴ showed the presence of the virus on up to 13% of bathroom, living room and kitchen sites. Most frequently contaminated were hand-contact sites such as bathroom taps, door handles, toilet flushes, liquid soap dispensers, nappy changing equipment and potties.

In this study, we compared the effectiveness of detergent- and disinfectant-based cleaning regimes typical of those that could feasibly be used to decontaminate the environment and reduce spread of NV from an infected source to a healthy recipient. Epidemiological evidence suggests that environmental spread from an infected person occurs by settling of aerosol particles on to contact surfaces, which are then touched by the hands, or by splashing or aerosol generation during toilet flushing, which spreads the virus to contact surfaces such as the toilet seat or flush handle.^{3,9-12} In applying a hygiene procedure, the aim is to prevent transmission of the virus from these surfaces via hands, cloths and other surfaces where they may be picked up by the clean hands of an uninfected person and cause infection, either by direct transfer from hand to mouth, or transfer by handling of ready to eat foods. As the infectious dose for NV can be very small, procedures were assessed on the basis of their ability to eliminate all detectable traces of the virus from the surface.

For the cleaning and disinfection experiments, surfaces contaminated with three levels of the NV infected faecal sample were used. The highest level (1 in 5 dilution of the faecal sample) was chosen to simulate surfaces that were directly soiled with infected faecal material, whereas the lower levels (1 in 10 and 1 in 80 dilutions) were intended to simulate surfaces that have become contaminated after secondary transfer via hands or cleaning cloths. In all cases, it was found that detergent-based cleaning with a cloth to produce a visibly clean surface was insufficient to eliminate NV contamination. These results are in agreement with the findings of Barker *et al.*²⁰ and Cogen *et al.*^{25,26} These workers showed that for elimination of bacteria from surfaces contaminated during handling of raw poultry using detergent-based cleaning, thorough rinsing of the surface in clean water is a crucial step (although for *Salmonella* spp., even cleaning with rinsing was insufficient).

In this investigation, we were able to show that handwashing with rinsing was satisfactory for elimination of NV from hands, but we reasoned

that for environmental surfaces likely to be involved in transmission of NV (door, tap and toilet flush handles, toilet seats, etc.), rinsing was not an option. With this in mind, we investigated whether rinsing the cloth after the initial wipe, and then rewiping the surface, could suffice as a 'rinsing step' but this was not the case. NV contamination remained on the surfaces. Our studies also showed that where the wiping cloth was used to wipe another surface, NV could be recovered from both the secondary surface and the hands of the person handling the cloth. This occurred regardless of whether the surface was wiped once or twice. Thus it is clear that detergent-based cleaning without adequate disinfection carries the risk of increasing rather than reducing the risk of infection transmission.

The results of our investigation support the current NV control guidelines, namely that in situations where there is significant risk of NV transmission, use of a disinfectant is recommended.¹⁶ However, the effectiveness of the disinfection procedure depends on a number of factors. It was found that where there was faecal soiling, although hypochlorite bleach at 5000 ppm produced a significant risk reduction, NV contamination could still be detected on up to 28% of surfaces. Increasing the contact time from 1 to 5 min appeared to have little effect. In order consistently to achieve a hygienic state, it was necessary to wipe the surface clean using a cloth soaked in detergent before applying disinfectant. This observation supports the recommendations made in the NV control guidelines which specify removal of any solid matter directly into a clinical waste bag, followed by cleaning with detergent and hot water using a disposable cloth and then disinfection with hypochlorite.¹⁶ In contrast, it was found that where surfaces were contaminated with NV-infected faecal suspension diluted to 1 in 10 and 1 in 80, treatment with a combined bleach/detergent formulation at 5000 ppm available chlorine, without prior cleaning, was sufficient to decontaminate surfaces. This suggests that for secondary contact surfaces that have become contaminated by transfer via hands and cloths, and where organic soiling is minimal, disinfection with bleach is satisfactory.

As tissue-culture methods are not available for NV, there is no direct evidence to support the use of a particular disinfectant for environmental decontamination. However, NV is a non-enveloped virus and is therefore unlikely to be inactivated by lipophilic compounds such as alcohols or quaternary ammonium compounds at levels used for disinfection of environmental surfaces.²⁷ A feline calicivirus has

been used as a surrogate for NV and found, in tissue culture-based studies, to be inactivated by hypochlorite solution at 5000 ppm.²⁸ For the present study, we used hypochlorite at 5000 ppm, which is the concentration used in many household surface cleaners.^{25,26} The RT-PCR assay has obvious limitations as it does not give any indication as to the viability and infectivity of the residual viral RNA. It is possible that a disinfection-damaged viron could release the viral RNA genome, producing a positive RT-PCR result. As RNA released from the viron is known to be degraded rapidly by RNases found widely in the environment, we speculate that the presence of detectable RNA indicates the presence of intact virus particles. However, damage caused to the viral protein capsid by hypochlorite is likely to render the virus non-infectious before degradation of the viral genome. Thus, RT-PCR is probably a conservative measure of virus survival and it is possible that if a tissue-culture method becomes available for NV, lower concentrations of bleach may be sufficient to inactivate the virus.

This study highlights the fact that detergent-based cleaning without adequate disinfection carries the risk of increasing rather than reducing the risk of infection transmission. Our results confirm that key elements in the control of NV are a combination of decontamination of the environment (particularly contact surfaces) and implementation of a thorough handwashing technique. Handwashing alone is unlikely to be effective if recontamination occurs via environmental fomites. This study has shown that fingers can both deposit and acquire NV when they come into contact with environmental surfaces. A similar study with rotavirus highlighted the vehicular role for hands in the spread of rotavirus infection.²⁹ It was argued that the roles of fomites and hands in rotavirus transmission are complementary and may be synergistic. Handwashing for 20 s is generally recommended for decontaminating hands to reduce cross-infection risks in hospitals and after visiting the toilet.³⁰ Using a thorough 1 min handwashing technique, we were able to remove NV from faecally contaminated hands to levels that gave negative RT-PCR assays. Due to limitations of the RT-PCR technology—in particular sampling volume—we cannot exclude the possibility that very low and yet infectious levels of virus remain on the fingers after thorough handwashing. For cleaning soiled surfaces after gastroenteritis incidents, the use of disposable latex gloves should be recommended. During outbreaks of viral gastroenteritis, effective decontamination of hand and environmental surfaces in hospitals, community facilities and domestic homes is clearly essential if the burden of

cross-infection is to be reduced. Raising awareness about the potential risks through information, training and implementation of effective control measures is a continuing challenge for all health and community workers. A clear message is required that emphasizes the need for appropriate hygiene measures after attacks of diarrhoea and vomiting. For surfaces where there is significant soiling with infected faecal or other material, this must include removal and disposal of soil before disinfecting with an appropriate compound. For other 'secondary' contact surfaces such as door and tap handles, use of a cleaner/disinfectant is considered satisfactory.

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