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Potential distribution of viable norovirus after simulated vomiting

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SUMMARY

Background: Vomiting is one way in which the body rids itself of harmful gastric contents rapidly. Whilst this process is generally beneficial for the emetic individual, it can pose significant infection control issues if they are infected with a highly communicable pathogen such as norovirus. It is not known how far norovirus could spread through vomiting while remaining viable, particularly in far-reaching droplets and splashes that might be missed during cleaning.

Aim: To identify the potential level of dissemination of viable norovirus after simulated vomiting.

Methods: This study used a system called 'Vomiting Larry' to simulate vomiting with infection medium containing the norovirus surrogate feline calicivirus (FCV) as a worst-case scenario for distribution and survival of viruses after simulated vomiting. Air and floor samples were taken after simulated vomiting, and analysed for viable virus via plaque assay. Analysis of covariance investigated differences in FCV concentration by sample volume and location.

Findings: Whilst viable virus was not isolated from any air samples taken after simulated vomiting, FCV concentrations of $\geq$10 plaque-forming units/mL were recovered from almost all samples taken from the floor (88/90). These included small droplets of fluid that travelled 3 m away from the vomiting system. There was evidence that FCV concentration depended on both sample volume and location.

Conclusion: This study suggests that norovirus can survive being ejected even within small far-reaching droplets at concentrations capable of eliciting infection. Such droplets could easily go unnoticed and be overlooked during cleaning, adding to the challenge of controlling norovirus outbreaks.

Introduction

For humans, in keeping with all other living creatures, to survive they must rid themselves of harmful substances such as toxins and pathogens. Vomiting is one way in which some mammals have adapted to rid themselves of these harmful

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substances rapidly. Whilst this process is generally beneficial for the emetic individual, vomiting (particularly projectile vomiting) can pose significant infection control issues if they are infected with a highly communicable pathogen such as norovirus (NoV). Electron microscopic studies have shown that as many as 3×10^7 NoV particles could be present in a 30-mL bolus of vomit [1]. In addition, work by Saetta and Quinton [2] found that volumes of vomitus fluid produced during emesis can vary between 0.4 and 1.35 L, which was discovered after 13 self-poisoned patients were treated with ipicacuanha [2]. This suggests that 1×10^9 NoV particles/L could be distributed into the immediate environment following a vomiting episode.

Transmission of NoV to new hosts could occur both during and after vomiting through direct contact, airborne and fomite routes. During emesis, droplets might land directly on to mucous membranes (eyes, nose and mouth) of those within close range of the emetic individual. If viruses contained within such droplets are ingested, they can proliferate in the gut and elicit infection. Spread of NoV via aerosols generated through vomiting, particularly projectile vomiting, could occur according to mathematical models derived from epidemiological data and supposition [3–5]. There is no evidence to suggest that NoV is capable of replicating in the human respiratory system, but the beating of respiratory cilia does cause the mucus coating and any entrapped particles to move towards the larynx, which is then either coughed out or swallowed. Therefore, inhaled NoV particles may be transferred from the respiratory system to the digestive tract, subsequently creating the potential for infection to establish within the gastrointestinal system.

The amount of environmental contamination from vomitus fluid has been identified with the use of a simulated vomiting system known as 'Vomiting Larry' [6]. Using 1 L of water containing a fluorescent marker as a worst-case vomitus simulant, this study highlighted that the main bulk of fluid covered an area of 1.92 m^2, but splashes and smaller droplets covered an area >6.6 m^2. Significantly, only the main bulk of the fluid and major splashes were visible under standard white lighting; smaller, more widespread droplets were difficult to identify without the use of ultraviolet light to visualize the fluorescent marker [6]. Consequently, transmission is most likely to occur after vomiting via hand-to-mouth contact after touching surfaces not recognized as being contaminated.

Whilst the previous study identified the spread of fluid after simulated projectile vomiting, it did not ascertain whether NoV could spread as far whilst remaining viable. The study described here used Vomiting Larry to identify the level of NoV survival during the simulated vomiting process and subsequent dissemination of viable virus using the common surrogate feline calicivirus (FCV).

Methods

Cell culture

Crandell Rees feline kidney epithelial (CRFK) cells were grown from a stock culture [European Collection of Authenticated Cell Cultures (ECACC), Public Health England, London, UK], and maintained in complete Eagle's minimal essential medium (EMEM; Sigma-Aldrich Corp., St Louis, MO, USA) containing 10% fetal calf serum (FCS; Fetalclone II, Hyclone Ltd, Cramlington, UK), 100 units/mL penicillin, 0.1 mg/mL streptomycin, 0.1 mM non-essential amino acids (NEAA; Sigma-Aldrich Corp.), and 2 mM GlutaMAX (Thermo Fisher Scientific Inc., Waltham, MA, USA). Cells were incubated in a humidified incubator at 37°C, 5% CO_2 and passaged twice weekly with trypsin EDTA 0.25% (Thermo Fisher Scientific Inc.).

Virus culture

CRFK cells were seeded at a density of 1×10^7 cells/flask in T175 flasks and incubated overnight (37°C, 5% CO_2) to achieve an 80% confluent monolayer. The culture medium was then removed and the cells were washed with phosphate-buffered saline (PBS; Lonza Group Ltd, Basel, Switzerland) solution without calcium or magnesium followed by a wash with virus infection medium (EMEM plus 2.5% FCS, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 2 mM and 0.1 mM NEAA). The virus infection medium was then removed, and 2 mL of infection medium containing FCV (strain F9, ECACC, PHE, UK) at a multiplicity of infection of 0.01 was added. The cells were incubated at 37°C, 5% CO_2 for 1 h to allow the virus to adsorb to the cells. During this time, the flask was rocked several times to ensure even viral adhesion across the monolayer. The 2 mL of infection medium containing any unattached virus was removed and the cells were washed with PBS. Infection medium (25 mL) was then added to the flask and incubated for two to three days (37°C, 5% CO_2) before harvesting the virus.

Cells were scraped from the base of the flask into the medium, and the cell/virus suspension was then transferred to a sterile 50-mL centrifuge tube (Gosselin UK Ltd, Manchester, UK). Three rounds of freeze–thawing were then undertaken to release virus particles from previously intact cells. Freezing was carried out by placing centrifuge tubes in a -70°C freezer for 1 h followed by thawing at 37°C in a water bath for 1 h. Cellular debris was removed by centrifugation (Heraeus Multifuge 35-R; Thermo Fisher Scientific Inc.) at 2000 rpm for 5 min at 4°C. The supernatant containing the virus was transferred to a fresh centrifuge tube before preparing 1-mL aliquots in cryovials (1.5 mL Nalgene; Thermo Fisher Scientific Inc.) for storage at -80°C. Virus concentration was calculated using a plaque assay.

Experimental set-up

Vomiting Larry was set up in a controlled atmosphere chamber (CAC) as described previously [6]. However, white rather than black plastic sheeting was used to cover the floor of the CAC to contrast the orange/red colour of the infection medium used here as the simulant gastric fluid to maximize potential spread of fluid and survival of NoV. A small section behind Vomiting Larry was left uncovered by the plastic sheeting to ensure that the room could be cleared of any virus aerosols using the CAC's remote air-handling system.

The simulated vomiting system was disinfected with Virkon (1% w/v; Thermo Fisher Scientific Inc.) followed by 70% ethanol prepared using ethanol absolute (Thermo Fisher Scientific Inc.) and water. The system was then rinsed with infection medium without virus to remove any residual disinfectant. The cylinder of the simulated vomiting system was filled with 1 L of infection medium containing 1×10^9 plaque-forming units (pfu)/L of FCV. The properties of vomitus are highly variable in volume,

consistency and chemical properties depending on how many times an individual has vomited over a relatively short period of time, what an individual has consumed, medication taken etc., which will subsequently alter the level of distribution and survival of NoV. Infection medium was used here not to simulate gastric fluid *per se*, but to offer FCV the best chance of survival whilst maintaining a worst-case scenario in terms of fluidity and, as such, maximum distribution of fluid. Samples (3×1 mL) were taken from this fluid and used as positive controls. These samples were used to confirm virus titre at source, immediately prior to vomiting simulation. The conditioned temperature and humidity of the CAC was confirmed before switching off the air-handling system and running the simulated vomiting system as described previously [6]. After experimentation, the CAC's air-handling system was switched back on to purge the room of aerosols for 30 min prior to re-entry into the chamber for sampling. The CAC facility and Vomiting Larry were decontaminated by fumigation using formaldehyde before and after each simulated vomiting trial to ensure any remaining FCV and any environmental micro-organisms present were inactivated prior to the start of each experiment.

Floor sampling

After simulated vomiting, 3×1 mL samples were taken at the site of the bulk spilled fluid (1.2 m in front of Vomiting Larry). Sterile sampling sponges (TS/15-B; Technical Service Consultants Ltd, Heywood, UK) were used to sample splashes at 12 predetermined locations across the floor (Figure 1). The locations were chosen based on observations from previous experiments carried out using ultraviolet fluorescent fluid [6]. Sponges were aseptically squeezed to release as much of the liquid sample as possible into 50-mL centrifuge tubes. The samples were then analysed by plaque assay. Floor sampling trials were conducted six times.

Additionally, three look-see samples were taken of small isolated droplets (~5-mm diameter) at 1, 2 and 3 m in front of Vomiting Larry using sterile swabs (Sterilin; Thermo Fisher Scientific Inc.). Each swab was immersed in 1 mL of virus infection medium in a 2.5-mL cryovial (Thermo Fisher Scientific Inc.) and gently rotated to dislodge the virus from the swab into the medium. The tips of the swabs were cut from the shaft, and the samples were refrigerated overnight to soak. The swabs were then removed taking care to extract as much medium as possible by pressing the swab against the side of the tube. To determine the concentration of viable virus, samples were analysed by plaque assay.

An additional set of experiments was undertaken to identify how much virus would actually be recovered from the floor using the swabs and sponges. This involved using sections of plastic sheeting with a 20-cm × 20-cm area marked out with masking tape. A known volume and concentration of FCV was applied via pipette within the allocated area before sampling and analysing as noted above. The volumes used were 50 μL, 0.5 mL, 1 mL, 1.5 mL and 2 mL of a 6.4×10^7 pfu/mL stock of FCV. These volumes were representative of the actual volumes collected during the main experiment. The 50-μL sample was collected using a swab, and the other volumes were collected using sponges. The numbers of viruses recovered were compared with the numbers of viruses in the equivalent volumes of stock. This was repeated three times.

Air sampling

Midget impingers (SKC Ltd, Blandford Forum, UK) were used to sample the air after simulated vomiting. Normally, midget impingers are used for the collection of chemicals and dusts from the air by bubbling air through a large volume of liquid to capture them. However, they have also proved useful for the isolation of bacteria [7] and viruses [8,9]. In this instance, it was likely that the concentration of virus recovered would be small, and would be diluted further by a large volume of liquid, and/or that the vigorous bubbling of the fluid would render some of the recovered virus particles non-viable. In either case, this might have led to a false-negative result via plaque assay. A smaller volume of liquid (750 μL) was therefore chosen, preventing overdilution of recovered virus particles.

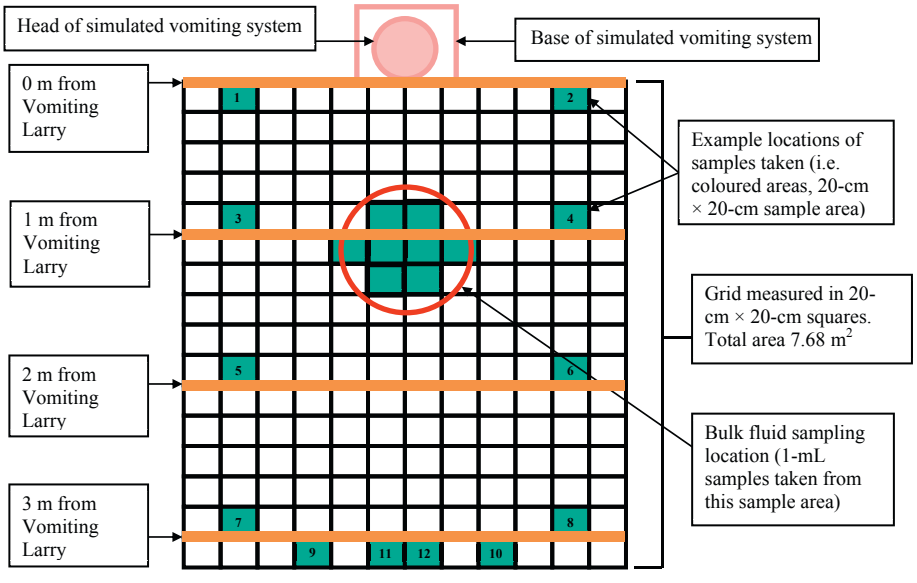

Figure 1. Grid layout of controlled air chamber and locations of samples analysed for the presence of norovirus.

While some evaporation losses occurred using this volume of liquid, it was found not to dry out completely or compromise the viability of the virus during the sampling process.

The impingers were set to sample air at 1.52 m above floor level (i.e. nose height of the simulated vomiting system). To prevent impaction and contamination from projectile vomited fluid, the impingers were set 1 m to the right of the vomiting system. Impingers were set to sample air at 0, 1 and 2 m away from the mouth of the system by connection with air pumps (B105; Charles Austen Pumps Ltd, West Byfleet, UK) via PVC tubing (10-mm outer diameter, 1.5-mm wall; Thermo Fisher Scientific Inc.). One impinger at each location was not attached to a pump, but remained in the chamber throughout the experiment and was used as a negative control (i.e. to test for accidental contamination that is not sampling related). Two other impingers were set up at each location to sample air at a rate of 1 L/min for 0–30 min and 30–60 min after simulated vomiting. Flow rate was set using a rotameter (flow range 0.3–3.0 L/min; SKC Ltd). All impingers contained 750 μL of infection medium to collect viruses from the sampled air.

Immediately after vomiting, a set of midget impingers was set running at a rate of 1 L/min for 0–30 min at a distance of 0, 1 and 2 m away from the mouth of the simulated vomiting system. At 30 min after vomiting, the first set of impingers was switched off and the second set was switched on and run for a further 30 min (i.e. 30–60 min after simulated vomiting). At 60 min after vomiting, the second set of impingers was switched off and the CAC air-handling system was switched on to remove any residual virus particles from the air. After a further 30 min, the room was re-entered and the outer surfaces of the midget impingers were wiped with 1% Virkon followed by 70% ethanol to kill and remove any attached virus from the outside of the impingers, avoiding cross-contamination of the sample within the impinger. The 750 μL of infection medium contained within the midget impingers was removed. Where the full 750 μL could not be recovered due to evaporation of the medium, the total volume was reconstituted to 750 μL using fresh infection medium. These samples were then analysed via plaque assay. Fumigation of the CAC facility and Vomiting Larry using formaldehyde was undertaken between each simulated vomiting trial to further prevent any NoV contaminant carryover. Air-sampling tests were repeated three times.

Plaque assay

Recovered samples were analysed to identify the numbers of viable viruses by means of a plaque assay. Six-well plates (flat bottom with lid; Sigma-Aldrich Co.) were seeded with 5×10^6 CRFK cells per well, contained within 3 mL of complete EMEM medium (containing 10% FCS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 2 mM GlutaMAX and 0.1 mM NEAA) and incubated at 37°C, 5% CO_2. Plates were gently rotated from side to side, back and forth for 30 s, and this action was repeated every 30 min over a 2-h period to ensure that the cells settled evenly. Cells were incubated overnight.

The medium was removed from the wells and the cells were washed with sterile PBS. The cells were then washed with infection medium. Dilution series of the viral samples were prepared using infection medium as the diluent. Infection medium was removed from the six-well plates, and aliquots of 1 mL of the diluted virus samples and 250 μL of the undiluted virus samples were transferred to the wells. One-millilitre aliquots of infection medium without virus were also used in one well of each six-well plate as negative process controls. Plates were gently rotated as described above to ensure even viral adhesion, and incubated for 1 h at 37°C, 5% CO_2 before removing the infection medium. The cells were then washed with sterile PBS to remove any unattached virus particles before adding 2 mL of overlay medium. The overlay medium contained pre-warmed 2× minimum essential medium (MEM) (without neutral red) (Thermo Fisher Scientific Inc.) containing 5% FCS, 200 U/mL penicillin, 0.2 mg/mL streptomycin, 4 mM GlutaMAX and 0.2 mM NEAA. This was mixed with 3% molten, low melting point (LMP) agarose (Thermo Fisher Scientific Inc.) at a ratio of 1:1. Once the overlay agarose had set, plates were incubated at 37°C, 5% CO_2 for three days.

The plaques were visualized by staining with neutral red dye as follows. A second overlay was prepared using equal parts of pre-warmed 2×MEM (without neutral red or additives) and 3% molten LMP agarose. To this, neutral red (3.3 g/L Sigma-Aldrich Co.) was added at a ratio of 39:1.2 agarose to neutral red, respectively. To each well, 2 mL of the molten overlay were added. Once set, the six-well plates were returned to the incubator at 37°C, 5% CO_2 for 2–4 h to allow plaques to become visible. The numbers of pfu per well were then counted visually, and the numbers of pfu/mL of the original samples were calculated.

Statistical analysis

Data collected from the floor samples were positively skewed and therefore summarized using median plus minimum and maximum numbers, rather than mean values. Analysis of covariance (ANCOVA) was used to determine whether the numbers of pfu/mL differed between the locations sampled (control, bulk or other floor) and between the volumes isolated

Table I
Fluid volume and viable feline calicivirus isolated after simulated vomiting

Location of samples	No. of samples taken	pfu/mL			Sample volume (mL)		
		Median	Minimum	Maximum	Median	Minimum	Maximum
Control	6	6.05×10^4	3.1×10^4	9.3×10^4	1.00	1.00	1.00
Bulk	18	7.25×10^4	2.7×10^4	1.6×10^5	1.00	1.00	1.00
Other floor location	72	350	0	1.36×10^6	0.80	0.50	3.60
Air	18	0	0	0	0.75[a]	0.75[a]	0.75[a]

pfu, plaque-forming units.
[a] Buffer in midget impinge.

per location (continuous measure). ANCOVA allows multiple factors to be investigated at the same time, and can include both categorical (i.e. location) and continuous (i.e. volume) independent variables. The result for location is therefore 'adjusted' for volume, and vice versa. Experiment number (categorical) was also included in the ANCOVA to adjust for any potential differences between experiments. The natural log was used to normalize pfu/mL for analysis. Where zero pfu/mL was observed (2/96 samples), half of the minimum non-zero observed pfu/mL was used instead, so that the log could be calculated. The underlying regression model of the ANCOVA was used to estimate the differences between locations and the estimated change in pfu/mL with increasing volume.

Results

Table I shows the volumes of fluid and concentrations of viable FCV recovered after simulated vomiting. Virus was not recovered from any of the air samples. Viable FCV was recovered from 98% (88/90) of all replicate floor samples across all locations. Concentrations were all ≥10 pfu/sample where viable FCV was recovered. The limit of detection (LoD) was 1 pfu/sample as samples of varying volumes were collected. Where samples collected exceeded 1 mL, the LoD was 1 pfu/mL. The volumes of fluid and concentrations of viable FCV recovered differed substantially between the locations sampled (Table I). Figure 2 shows a post-simulated vomiting image under ultraviolet light from previously published research [6] overlaid with the locations sampled in this study. Volumes of fluid and numbers of viable viruses recovered from each location helped to visualize the impact of small droplets and splashes. Highest concentrations of FCV were recovered from areas sampled closest to the bulk of the fluid after simulated vomiting: locations 3, 4, 5 and 6 (as noted in Figure 2). The look-see ~5-mm diameter droplets sampled at 1, 2 and 3 m from Vomiting Larry recovered 8, 25 and 17 pfu of FCV, respectively.

Table II
Analysis of covariance for the numbers of viable virus particles isolated from floor samples

	Sum of squares	Degrees of freedom	Mean square	F-statistic	*P*-value
Model	802.37	8	100.30	21.56	<0.001
Location	530.88	2	265.44	57.06	<0.001
Volume	359.30	1	359.30	77.23	<0.001
Experiment	12.43	5	2.49	0.53	0.750
Residual	404.75	87	4.65		
Total	1207.12	95	12.71		

Table II shows the results of the ANCOVA for the floor samples, which was used to allow multiple factors to be assessed simultaneously including both categorical (i.e. location) and continuous (i.e. volume) independent variables. There was strong evidence of a difference in the pfu/mL both by location and by sample volume (both $P<0.001$). Statistically, there was no significant difference between bulk samples and controls ($P>0.10$). However, bulk samples were significantly greater than samples from the other floor locations (i.e. locations 1–12, see Figures 1 and 2). On average, the concentration of FCV in bulk samples tended to be about 250 times greater than other floor samples (estimated ratio = 251, 95% confidence interval 80–784, $P<0.001$). The pfu/mL tended to increase by ~38% for each 0.1-mL increase in sample volume (estimated ratio = 1.38, 95% confidence interval 1.28–1.48).

As might be expected, the numbers of viruses surviving and retrieved in process controls were considerably lower than those applied to the plastic sheeting during the recovery control tests (Table III). Between 0.32 and 3.95% of the applied numbers of viruses were recovered.

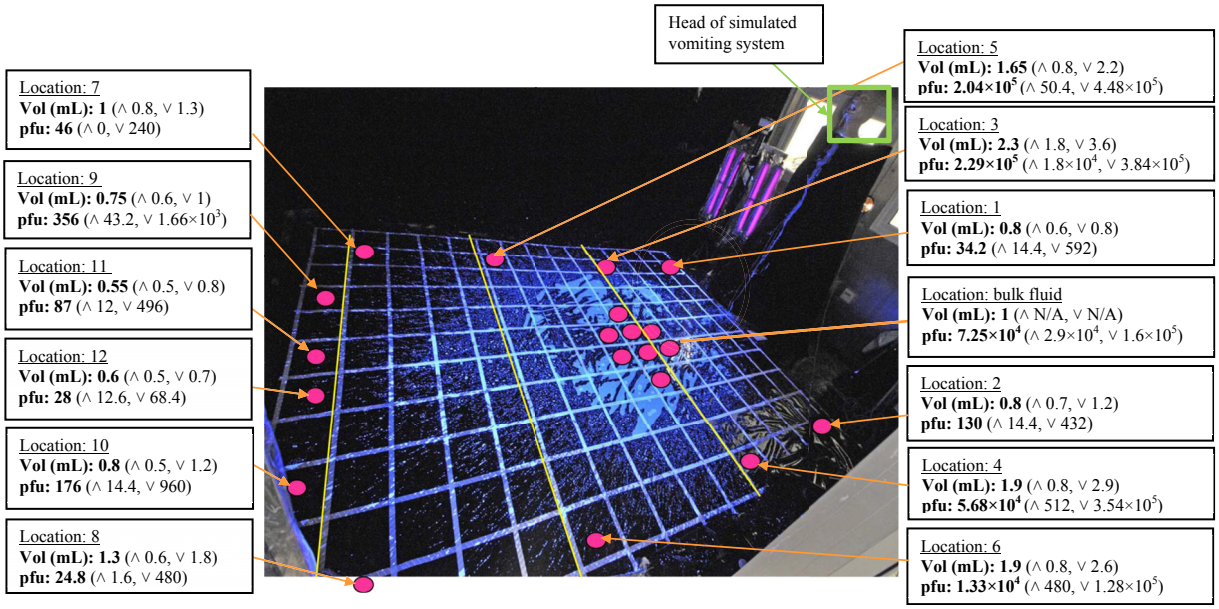

Figure 2. Observed pattern of fluid distribution after simulated vomiting, highlighting median volumes of fluid and concentrations of viable virus [plaque-forming units (pfu)/sample] recovered from the chosen sample locations (∧ = minimum recovered, ∨ = maximum recovered). Total number of samples taken from location bulk fluid was 18. Total number of samples taken from all other locations was 6.

Table III
Percentage virus recovery from swabs (50 μL only) and sponges compared with the numbers applied to plastic sheet surface

Sample volume	Mean recovery compared with that applied to the surface (%)
50 μL	0.322 (±0.127)
0.5 mL	4.125 (±0.459)
1 mL	4.618 (±1.084)
1.5 mL	4.074 (±0.637)
2 mL	3.958 (±0.966)

Discussion

Viable virus was not recovered from any of the air samples taken after simulated vomiting. There are a number of possible explanations for this. First, aerosolized FCV might not survive the drying process when airborne; second, the amount of viable virus might be below the level of detection (1:250 μL) for the plaque assay; third, the Midget impinger sampling method (not well validated for collection of viral aerosols) may have been unable to adequately capture or isolate airborne virus particles; or fourth, aerosols are not generated. Lack of aerosol generation seems unlikely, given that the infectious dose of NoV could be as low as 10 virus particles, and the volume of fluid (previously noted up to 1.35 L) and concentrations of NoV contained within it (1×10^9viruses/L) likely to be emitted during projectile vomiting of an infected individual are considerable. Further investigations are required to establish the potential for aerosols containing viable viruses to be produced during emesis.

Virus was recovered from almost all (88/90) of the samples taken from the floor of the CAC, even those furthest away (3–3.2 m) from the system. Quantities recovered were considered to be capable of eliciting infection (i.e. ≥10 viruses), demonstrating that NoV survives well during the process of being ejected. These data also concur with the views of Cheesbrough [10] and Lamhoujeb [11] that NoV is robust and can survive in the environment after vomiting. Differences in the numbers of viable viruses isolated were dependent both on the location sampled and on the recovered volume. As might be expected, the locations from where the greatest volumes of fluid were recovered were those closest to, and within, the main bulk of the spilled fluid. The number of viruses contained within samples of bulk fluid was much greater (estimated 250 times/mL more) than those isolated from other floor samples. From this study, it was estimated that, on average, the pfu/mL of viruses recovered increased by approximately 38% for each 0.1-mL increase in volume recovered. Several factors may account for this trend. It is possible that the virus particles aggregated, causing them to remain within the bulk fluid; virus particles might be protected to a greater extent within larger fluid volumes due to the proteinacious components of the infection medium and/or being less exposed to drying; larger fluid volumes might cushion virus particles, resulting in less virus shearing; smaller droplets may be more likely to be a result of rebound splashes, meaning that virus particles will have experienced additional shearing; and sampling of bulk fluid with pipettes may have been more efficient than the sponge method that was used for smaller volumes.

This study has a number of other limitations. The medium used as the simulant vomitus fluid offered FCV the best chance of survival whilst maintaining a worst-case scenario in terms of fluidity, and as such, maximum distribution of fluid. In reality, the properties of vomitus are highly variable in volume, consistency and chemical properties depending on how many times an individual has vomited over a relatively short period of time, what an individual has consumed, medication taken etc., which will subsequently alter the level of distribution and survival of NoV. FCV used as a surrogate for human NoV is thought not to be as robust as human NoV; thus, human NoV survival may be greater than estimated here. Potential movement of fluid between the 20-cm × 20-cm sections during sampling may have caused some inaccuracy in exact volume collection. Finally, sponges were squeezed to release fluid and virus particles, meaning that many virus particles remained trapped in the sponges (~96% based on the recovery control tests) after sampling. Centrifugation of the sponges might have allowed an increased number of viruses to be eluted and could have increased assay sensitivity. Despite these limitations, this study shows the potential for NoV infectious doses to be widely disseminated during vomiting.

In conclusion, small droplets of fluid travelled >3 m in front of the vomiting system, emphasizing that NoV can survive being ejected even in small far-reaching droplets. Based on recovery control tests, various volumes of viral stock applied to plastic sheeting and sampled using swabs and sponges recovered merely ~0.3% of the applied viral concentration from the swabs and ~4% from the sponges. Taking location 12 as an example, an average of 28 pfu were recovered via a sponge at this position 3–3.2 m from Vomiting Larry. If this equates, as is suggested by the recovery control tests, to 4% of the total number of viruses known to be present on the surface, the actual number of viruses present in that location was likely to be ~700. This information is important in terms of infection prevention and control, as droplets and splashes that are missed during cleaning and disinfection are likely to contain numbers of viruses well in excess of that required to elicit infection (≥10 pfu/mL), adding to the challenge of controlling NoV outbreaks.

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Conflicts of interest
None declared.

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References

[1] Caul EO. Hyperemesis hiemis — a sick hazard. J Hosp Infect 1995;30(Suppl.):498–502.

[2] Saetta JP, Quinton DN. Residual gastric content after gastric lavage and ipecacuanha-induced emesis in self-poisoned patients: an endoscopic study. J R Soc Med 1991;84:35—8.
[3] Sawyer LA, Murphy JJ, Kaplan JE, Pinsky PF, Chacon D, Walmsley S, et al. 25- to 30-nm virus particle associated with a hospital outbreak of acute gastroenteritis with evidence for airborne transmission. Am J Epidemiol 1988;127:1261—71.
[4] Marks PJ, Vipond IB, Regan FM, Wedgwood K, Fey RE, Caul EO. A school outbreak of Norwalk-like virus: evidence for airborne transmission. Epidemiol Infect 2003;131:727—36.
[5] Leung T, Leung TF, Lai RW, Chan PK, Hon EK, Ng PC. Infection control for norovirus gastroenteritis outbreak in acute open-designed paediatric ward. Acta Paediatr 2006;95:581—6.
[6] Makison Booth C. Vomiting Larry: a simulated vomiting system for assessing environmental contamination from projectile vomiting related to norovirus infection. J Infect Prevent 2014;15:176—80.
[7] Tyler ME, Shipe EL. Bacterial aerosol samplers: I. Development and evaluation of the all-glass impinger. Appl Microbiol 1959;7:337—49.
[8] Snyder JC, Wiedenheft B, Lavin M, Roberto FF, Spuhler J, Ortmann AC, et al. Virus movement maintains local virus population diversity. Proc Natl Acad Sci USA 2007;104:19102—7.
[9] Makison Booth C, Clayton M, Crook B, Gawn JM. Effectiveness of surgical masks against influenza bioaerosols. J Hosp Infect 2013;84:22—6.
[10] Cheesbrough JS, Barkess-Jones L, Brown DW. Possible prolonged environmental survival of small round structured viruses. J Hosp Infect 1997;35:325—6.
[11] Lamhoujeb S, Fliss I, Ngazoa SE, Jean J. Evaluation of the persistence of infectious human noroviruses on food surfaces by using real-time nucleic acid sequence-based amplification. Appl Environ Microbiol 2008;74:3349—55.