
Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis

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SUMMARY

A protracted outbreak of Norwalk-like virus (NLV)-associated gastroenteritis occurred in a large hotel in North-West England between January and May 1996. We investigated the pattern of environmental contamination with NLV in the hotel during and after the outbreak. In the ninth week, 144 environmental swabs taken from around the hotel were tested for NLV by nested RT-PCR. The sites were categorized according to the likelihood of direct contamination with vomit/faeces. The highest proportion of positive samples were detected in directly contaminated carpets, but amplicons were detected in sites above 1.5 m which are unlikely to have been contaminated directly. The trend in positivity of different sites paralleled the diminishing likelihood of direct contamination. A second environmental investigation of the same sites 5 months after the outbreak had finished were all negative by RT-PCR. This study demonstrates for the first time the extent of environmental contamination that may occur during a large NLV outbreak.

INTRODUCTION

Norwalk-like viruses (NLVs, also known as SRSVs) are generally recognized to be the leading cause of outbreaks of diarrhoea and vomiting in the UK [1]. A typical case is characterized by sudden onset of nausea with projectile vomiting and watery diarrhoea, which resolves within 72 h. The combination of high viral load ($> 10^6$ particles/ml) in vomit and faeces, low infectious dose and lack of long-term immunity following previous infection accounts for the high secondary attack rate characteristic of NLV outbreaks. Contaminated food, aerosol and direct contact are believed to be the principal routes of transmission of NLV [2]. The role of fomites is less clear. While outbreaks in hotels and cruise ships in

which recurrent waves of infection occur in successive cohorts of guests suggest that environmental contamination may occur [3–5], direct evidence for this is lacking.

Several guidelines recommending measures to control outbreaks have been published and these include thorough environmental cleaning, changing curtains and steam cleaning carpets [6]. These recommendations are empirically based and the importance of contamination in particular environmental sites is unknown.

The development of a broadly reactive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for NLV, capable of detecting minute quantities of viral RNA [7, 8], provides a method for environmental sampling of this uncultivable group of viruses [9] and the possibility of directly addressing this issue. In

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this study we have examined the scale of environmental contamination in a large hotel during an extended outbreak of NLV infection using RT-PCR.

METHODS

Description of outbreak

The outbreak occurred in a large hotel (500 beds) in North-West England between January–May 1996. Over the winter period, the hotel lets rooms for 3-day (Monday–Wednesday) or 4-day (Thursday–Sunday) ‘mini-breaks’. Guests who had arrived on 15 January 1996 became unwell while still resident in the hotel with typical NLV symptoms. Three of six faeces samples collected from guests were positive for NLV by electron microscopy. The subsequent course of the outbreak is shown in Figure 1. This epicurve is based on cases of diarrhoea and/or vomiting occurring among staff or guests which were reported to the hotel management between 15 January 1996 and 24 May 1996. Cases occurring among guests after their departure have not been included.

The majority of cases (77%) among the staff occurred during the first three mini-breaks. The number of cases among guests fluctuated widely over the next 12 weeks until 15 March 1996 when the hotel was closed for a deep clean. A total of 850 of 4291 guests staying at the hotel between 15 January 1996 and 15 March 1996 developed diarrhoea and/or vomiting. The attack rate among guests in different mini-breaks varied from 2.2 to 39.1% with a mean of 19.8%. Many guests were elderly and were sometimes unable to reach toilet facilities before vomiting.

Initial investigations failed to identify any high risk foods such as uncooked shell fish, and no associations with any particular meals or food items were noted on examination of menu based questionnaires administered to available guests with recent NLV symptoms in the first three mini-breaks. A formal case control study was not undertaken due to logistic problems. No serious lapses of hygiene were found on an inspection of the kitchens.

Initial control measures included procedures to avoid any contact between consecutive groups of guests in the foyer on change-over days, removal of non-cooked food items from the menu and the formation of a cleaning team who were rapidly mobilized following an episode of contamination in a public area. This had no measurable impact on the outbreak and the hotel was closed on the 15 March 1996. While closed, the hotel was thoroughly cleaned;

hard surfaces with warm water and detergents and carpets by shampooing followed by vacuum cleaning. Disinfectants were not employed due to concern that the carpets and soft furnishings would have been damaged. The hotel re-opened after 1 week on 22 March. Cases of NLV rapidly increased again peaking in a mini-break from the 29 March to 1 April in which 92 of 226 (40.7%) were affected. After this, the attack rate diminished with no further clinical cases after 28 June 1996.

Faecal samples

Faecal samples from four patients, two in the initial wave in January 1996 and two from cases occurring in mid-March were selected for testing by RT-PCR. These had previously been shown to contain NLV by electron microscopy and had been stored at 4 °C prior to PCR testing. Seventeen faecal samples collected from 13 outbreaks of gastroenteritis occurring at hospitals, nursing homes and at a school in Lancashire between January and March 1996 were also tested by RT-PCR/sequencing in order to compare strains circulating in the local community with that associated with the hotel outbreak.

Environmental samples

On 15 March 1996, prior to cleaning, environmental samples were collected by surface wiping an area of approximately 5 × 5 cm with a cotton tipped swab. The tip of the swab was moistened in virology transport medium prior to sampling. Swabs were sent to the Central Public Health Laboratory (CPHL) in sufficient transport medium to keep the swab moist during transit (approximately 100 µl).

A total of 144 swabs were collected from a range of sites within the hotel. These were ranked into eight categories (Table 1). The hotel management identified eight areas of carpet where guests had vomited within the 72 h prior to sampling (Category 1). These areas had all been cleaned with water and detergent followed by vacuuming and appeared clean at the time of sampling. Another 12 areas of carpet with no definite record of direct contamination with vomit were sampled (Category 2). Samples from within the toilet area, are divided into those likely to be directly contaminated by vomit or diarrhoea (Category 3) and those without direct contamination in which hand transfer is the likely route of contamination (Category 4).

Outside the toilet areas, samples (other than those

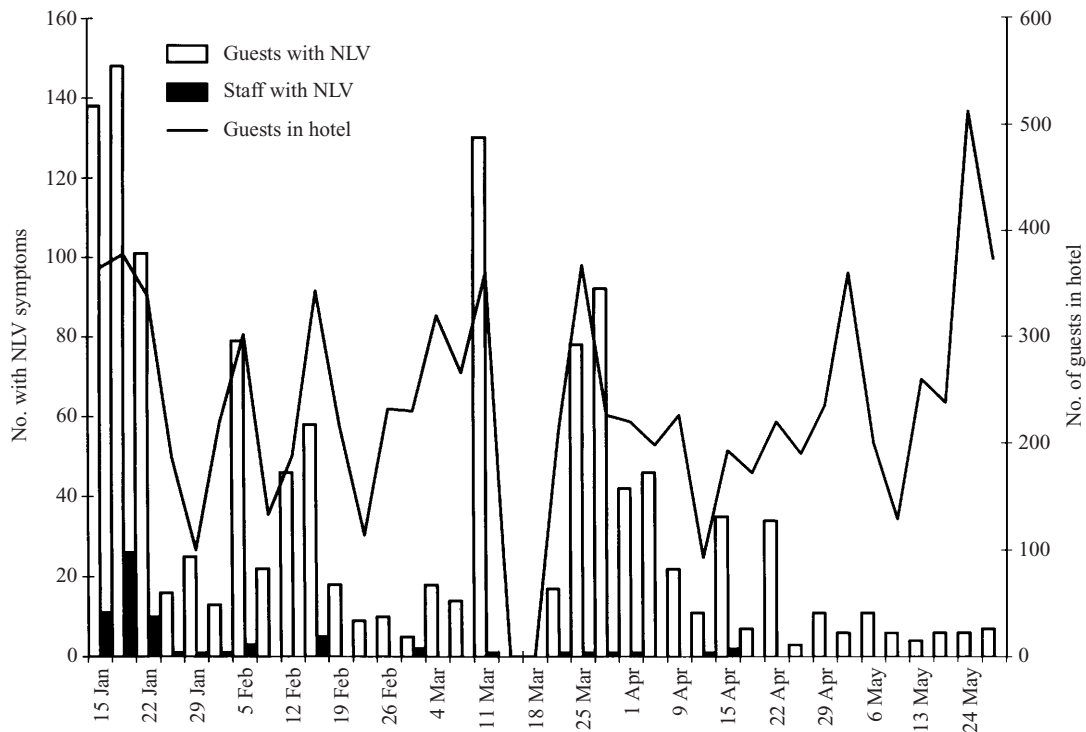


Fig. 1. Illness compatible with NLV among guests and hotel staff, January–May 1996. The number of guests on each mini-break, number falling ill while still in the hotel and the number of cases among hotel staff are recorded.

Table 1. Results of RT–PCR on environmental swabs from the hotel by site of collection categories

Category	Site category	RT–PCR results on environmental swabs	
		March 1996 Pos/total (%)	October 1996 Pos/total
1	Carpet (known recent vomit)	5/8 (62)	0/8
2	Carpet (no known recent vomit)	9/12 (75)	0/20
3	Toilet rims or seats	8/11 (73)	0/11
4	Toilet handles, taps, basins and surfaces	13/33 (39)	0/33
5	Horizontal surfaces (outside toilet) below 1.5 m, e.g. tables, ledges	11/29 (37)	0/29
6	Horizontal surfaces (outside toilet) above 1.5 m, e.g. mantle piece, light fittings	6/12 (50)	0/12
7	Frequently handled objects, phones, door handles, etc.	7/29 (24)	0/29
8	Soft furnishings, cushions, curtains, etc.	2/10 (20)	0/10
	Total	61/144 (42)	0/144

from carpets) were collected from a wide range of sites, including table and counter tops, dado rails, mantelpieces, tops of wardrobes, light fittings, switches, telephones and soft furnishings. These have been categorized into; hard horizontal surfaces below 1.5 m which may have been directly handled (Category 5); hard horizontal surfaces above 1.5 m of which direct handling is unlikely (Category 6); objects likely to be handled frequently such as door knobs, telephones, TV remote controls (Category 7) and soft

furnishing such as cushions or curtains (Category 8). Repeat samples from all sites were collected in October 1996, 5 months after the end of the outbreak.

RT–PCR

RNA extraction from faecal samples and environmental swabs

RNA was extracted from 100 μ l faecal extract using a modification of the ‘Boom’ method as previously

described [7]. The environmental swabs were processed similarly except that 900 μ l guanidinium isothiocyanate lysis buffer were added directly to the swab container. After thorough mixing, the swab was carefully removed from the container and discarded. The lysis buffer was removed from the swab container to an Eppendorf tube, spun in a microfuge for 1 min and 10 μ l silica particle suspension added. Adsorption of the RNA to the silica and subsequent washing and elution stages were as for the faecal samples. Complementary DNA (cDNA) was generated from the extracted RNA using random hexamers and MuMLV reverse transcriptase. This cDNA was used as template for each two PCRs; (i) Direct single-round amplification with the Ni/E3 primer pair [7], (ii) Nested RT-PCR with first round amplification with primers G1/G11/31 [8]. After 30 cycles, 1 μ l first-round mix was transferred for PCR with the nested primer pair Ni/E3 [8].

The amplicons from both the direct Ni/E3 PCR and the nested Ni/E3 PCR were analysed by electrophoresis in agarose gels. Amplicons of the correct size (113 bp) were confirmed to be NLV by Southern blot hybridisation with NLV-specific probes [7].

The nested RT-PCR was demonstrated to be 100-fold more sensitive than the single-round RT-PCR for the NLV strain associated with this outbreak (data not shown).

NLV strain characterization

PCR amplicons were separated from unincorporated nucleotides and primers using Chromaspin 100-TE spin columns and were sequenced using an ABI Taq FS cycle sequencing kit and an ABI automated sequencer. Sequence data were analysed using SeqED and DNASTar analysis packages.

RESULTS

Faecal samples

Four faecal samples collected from the hotel outbreak were positive for NLV by RT-PCR. Nucleotide sequences from all amplicons were identical, which showed that a single strain had been the cause of both the January and March incidents. Phylogenetic analysis showed that this strain was most closely related to Grimsby virus [10] with 97.5% nucleotide sequence identity within the intra-primer region. Faecal samples

from 10 of 13 contemporaneous outbreaks in the North West of England were positive by RT-PCR. Amplicons obtained from eight samples were suitable for sequencing, of which six were shown to be closely related (> 95% nucleotide identity within the 76 bp intra-primer region) to the strain associated with the hotel outbreak. This indicates that this strain was circulating widely in the community at the time of the hotel outbreak.

Environmental swabs

The results are expressed as first-round RT-PCR positive and nested RT-PCR positives. Six environmental swab samples were positive by direct first round RT-PCR, five of which were taken from carpets, and one from a toilet rim. By nested PCR, 61 (42%) of the 144 swabs were positive for NLV RNA. Table 1 shows a trend of diminishing frequency of RT-PCR positivity across the categories, which broadly correlates with the likelihood of direct contamination. Sites in all categories yielded RT-PCR positive swabs.

None of the 144 samples collected in October was positive for NLV RNA by nested RT-PCR.

DISCUSSION

Prolonged NLV outbreaks of this type have been recorded in other large institutions and cruise ships [5, 11] and this is the largest documented hotel outbreak. The ascertainment of the epidemiological data is imperfect, since it is reliant on self reporting by guests to the hotel reception desk. No attempt was made to exclude cases of diarrhoea or vomiting due to other causes nor cases presenting within the incubation period (15 h) of arriving at the hotel. While these factors may result in over reporting, it is more likely that substantial underreporting has occurred by not including any cases presenting within 36 h after leaving the hotel. While many such cases were reported to the hotel management they would not have contributed to the environmental contamination within the hotel.

Factors that may have contributed to the size of the outbreak include: the very rapid turnover of guests; the high level of occupancy; the advanced age and often some degree of disability among guests. The reliance on natural (open window) ventilation in most of the hotel and the problem this poses for maintaining

a comfortable temperature during the winter season may be an additional factor. The most remarkable features of this outbreak are its long duration and wide variation in attack rate between successive cohorts of guests.

The possible routes of transmission between successive cohorts of guests include a continuously contaminated food or water, direct contact or droplet spread between successive cohorts of guests, spread from hotel staff with long-term carriage of NLV, successive re-introduction of NLV by new guests or contact with contaminated fomites within the hotel environment.

These routes are clearly not mutually exclusive and it is likely that each may have played a role at some point in the outbreak. However, some estimate of the relative importance of each route can be deduced from epidemiological investigations and the pattern of the outbreak. Each will be considered in turn.

The possibility of contaminated food was investigated by examining kitchen hygiene and food histories of affected guests in the first two waves of the outbreak. No deficiencies in food handling practices were identified nor was there any clear association found with the consumption of any particular food. There were no reports of illness among catering staff while at work. High-risk foods such as raw shellfish were not served in the hotel and salads and cooked shellfish were withdrawn from the menu after the first wave of infection.

Measures to reduce direct contact between successive cohorts of guests by minimizing any mingling of departing and arriving guests were instigated after the third affected mini-break. This had no measurable impact on the course of the outbreak. Staff were affected during the first 2 weeks of the outbreak and cases were infrequent thereafter. Staff turnover was low and it is likely that the majority of staff would have developed immunity to this strain within the first month of the outbreak. While prolonged asymptomatic excretion among staff with subsequent spread to guests is a theoretical possibility, it is not supported by current knowledge of the duration of NLV excretion in humans.

The possibility of successive reintroduction of NLV into the hotel by guests incubating the illness on arrival cannot be completely discounted. During the first 3 months of 1996, numerous outbreaks of NLV were reported from hospitals, schools and nursing homes in North West England. However, the incubation period for NLV is short (12–60 h) and only

three other outbreaks of diarrhoea and vomiting suggestive of NLV were reported among the many hotels in the area. Moreover, the study outbreak persisted into May by which time reports of general community outbreaks of NLV had fallen to the average background level.

The final route, contact with contaminated fomites, appears to have played an important role in maintaining this outbreak. While transmission from fomites is likely to be inefficient compared with highly infectious aerosols generated by vomiting, it would explain the link between successive cohorts of guests. While infection from fomites directly may result in only a few cases, these cases may then cause a large number of secondary cases amongst other guests, resulting in a large wave of infection. A critical factor is likely to be the time and location at which these initial fomite derived cases become unwell: vomiting in a bedroom has a much lower risk of causing multiple secondary cases than in a busy public area. This pattern of a continuing background of cases in each mini-break with superimposed peaks fits well with that observed in this outbreak.

The rapid recrudescence of the outbreak when the hotel was re-opened after cleaning in late March could be explained by the immediate re-introduction by a guest incubating NLV at the time of arrival at the hotel. However the re-infection of guests from the environment with subsequent amplification by cross-infection between clinically affected guests seems more plausible.

While environmental contamination has been suggested as an important factor in outbreaks on epidemiological grounds, this is the first utilization of a nested RT-PCR to demonstrate the extent of environmental contamination with NLVs that can occur during such outbreaks. The uniformly negative results on the repeat sample survey confirm the specificity of these findings.

All the samples yielding a positive result on first round PCR were collected from sites likely to have been directly contaminated such as carpets or toilet rims. Positive carpet swabs were obtained after cleaning and in all cases were collected from carpets that appeared clean at the time of sample collection, indicating that standard carpet cleaning with detergent followed by daily vacuuming will not remove all virus. Samples positive on second round PCR were obtained from a wide variety of sites and were just as likely to be positive if collected from a high horizontal surface, very unlikely to have been touched, as they

were from items likely to be handled such as telephones, light switches or door knobs. This suggests that airborne dissemination occurs and virus persists in areas unlikely to be cleaned with any frequency.

An important question that cannot be answered with certainty is the extent to which a positive signal represents RNA not associated with viable virus. While it is possible that some of the positive PCR results represent non-infectious virus, NLVs have an ssRNA genome which is susceptible to RNAses found widely in the environment and it is likely therefore that positive signals are associated with virus particles.

Some previously reported prolonged outbreaks have only been successfully curtailed with extensive control measures including thorough environmental cleaning [3, 12]. We have not been able to establish the relative importance of contamination at different sites. The wide variety of sites yielding positive results offers infection control teams little assistance in targeting key areas for decontamination but the relatively high levels of RT-PCR signal found in carpets suggests that these should be a priority. The capacity of carpets to harbour viable virus for up to 12 days has been recently suggested [13]. Steam cleaning of carpets, which cannot tolerate hypochlorite, has been recommended as the most appropriate means of decontamination [14]. While formal evidence that this is superior to wet shampooing, steam cleaners were not used during the closure and this may, at least in part, explain the continuation of the outbreak after re-opening. This study suggests that the nested RT-PCR assay for NLVs, in addition to demonstrating the extent of environmental contamination, may provide a means to formally evaluate cleaning and decontamination methods including steam-cleaning and thus to develop procedures to limit the time course of NLV outbreaks in semi-closed institutions.

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