
Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant

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SUMMARY

An outbreak of gastroenteritis followed a meal in a large hotel during which one of the diners vomited. The clinical features of the illness suggested Norwalk-like virus (NLV, small round structured virus) infection, and this was confirmed by electron microscopy and reverse transcriptase polymerase chain reaction (RT-PCR) of stool samples. Further characterization of the virus by nucleotide sequence analysis of the PCR amplicons revealed identical strains in all the affected individuals. The foods served at the meal could not be demonstrated to be the cause of the outbreak. Analysis of attack rates by dining table showed an inverse relationship with the distance from the person who vomited. No one eating in a separate restaurant reported illness. Transmission from person-to-person or direct contamination of food seems unlikely in this outbreak. However, the findings are consistent with airborne spread of NLV with infection by inhalation with subsequent ingestion of virus particles.

INTRODUCTION

Norwalk-like viruses (NLVs) are a group of closely related and highly infectious viruses which were first reported following an outbreak of gastroenteritis in Norwalk, Ohio in 1972 [1]. They are 32 nm viruses with an amorphous surface and ragged outline and a buoyant density of 1.36–1.41 g/cm³ [2]. NLVs have the ability to cause outbreaks of gastrointestinal infection characterized by vomiting which is often sudden and projectile [3], as well as diarrhoea. Human NLVs have no known reservoir outside of man [4] although animal NLVs have been reported recently [5, 6].

In a review of UK outbreaks from 1992 to 1994 [4] the mean incubation period for 25 outbreaks was 35 h (range of medians 11–48 h). The mean duration of illness (23 outbreaks) was 42 h and the median attack rate (35 outbreaks) was 48% (range 4–100%). No

deaths were reported. Particular food items were suspected in only 24 of 41 outbreaks reported as foodborne with oysters accounting for 8 of the outbreaks. In 11 of the foodborne outbreaks the suspected food vehicle was served raw. There was no clear seasonal pattern but a quarter of the outbreaks occurred in November and December.

The faecal/oral route of transmission of infection is important in secondary spread of NLVs but vomitus also represents a major source of infection. It has been estimated that over 30 million virus particles can be liberated during vomiting, compared to an infectious dose of 10–100 particles [8]. Transmission from vomitus has been described in an outbreak in a hotel after a kitchen assistant vomited into a sink which was later used to prepare potato salad [9]. Airborne spread of the virus has also been proposed. Respiratory spread is unlikely as no evidence has been found of replication of NLV in respiratory mucosal cells. The contribution to secondary spread by airborne trans-

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mission may be important through the inhalation and subsequent swallowing of aerosolized virus and is more likely if there has been projectile vomiting in a confined space. Transmission of NLV by airborne droplets was implicated in a gastroenteritis outbreak on a cruise ship [10]. Index patients who had vomited in their cabins were more likely to have had cabin mates who subsequently became ill than were index patients who had not vomited. An outbreak of acute gastroenteritis in a hospital in Toronto associated with NLVs has been reported with evidence for airborne transmission [11]. However, studies so far have not excluded hand/mouth transmission from environmental contamination [12]. In this report we describe the first detailed account of an outbreak of NLV infection associated with airborne transmission arising from a diner who vomited during a meal.

THE OUTBREAK

Six parties of diners, totaling 126 people, attended an evening dinner at a large hotel on Monday, 7 December 1998. In addition a number of hotel residents and casual diners were using a restaurant in a separate room on that evening. At 8.30 p.m., during the meal, a lady in one party vomited onto the polished wooden floor. The vomiting was not projectile and none of it was thought to have made contact with the table. She had not been ill prior to the meal and had little warning that she was going to vomit. The vomitus was rapidly cleaned up by one of the waiters with a mop and disinfectant and the meal continued.

On the morning of Thursday, 10 December 1998 the organizer of the largest party contacted the local Environmental Health Department and reported that she was aware of 9 members of her party of 45 who had become ill with vomiting, diarrhoea and abdominal pain, mostly with an onset approximately 36 h after the meal. That afternoon a further report was received from another party that a number of their members had become ill following the meal. The illness lasted approximately 24–48 h in most people.

Following a previous kitchen inspection the Environmental Health Department was already working closely with the hotel to improve standards of kitchen hygiene. Coincidentally a visit had been made on the afternoon of 7 December, when it was felt that good progress had been made by the hotel. No staff in the kitchen or restaurant had reported any illness on or prior to 7 December, but one member of the Dining

Room staff became ill on 8 December with an illness similar to that of the diners which lasted 24 h. No other members of staff became ill.

METHODS

Epidemiological investigation

Environmental Health Officers started interviewing diners on 10 December using a structured questionnaire by telephone or personal interview. A cohort study was conducted to describe the epidemiology of the outbreak and to determine its cause. The questionnaire requested details of food eaten and the time and symptoms of any subsequent illness. Data from the questionnaire were analysed using Epi-Info version 6.04b (Centres for Disease Control, Atlanta) to calculate relative risks for all the foods that were served and attack rates by table. Those with symptoms, including the lady who vomited during the meal, were asked to submit stool specimens, although most had recovered by the time they were contacted. The hotel was visited, the kitchens inspected and the management interviewed on Monday, 14 December. A plan of the dining areas was made and records examined to enable contact tracing and case finding interviews of the other parties at the function.

Laboratory investigations

Stool samples were requested from diners who had been ill and tested for bacterial pathogens. For virological investigations, the faecal specimens were emulsified as approximately 10–20% suspensions in minimal essential medium (Eagle's) and the same sample used for solid phase immune electron microscopy (SPIEM) and reverse transcriptase polymerase chain reaction (RT-PCR).

Solid phase immune electron microscopy

NLVs were captured onto formvar/carbon grids coated with anti-human IgG and human convalescent serum as described previously [13] and then viewed using a Philips 420 electron microscope ($\times 62\,500$).

RT-PCR and DNA sequencing

Diagnostic RT-PCR was performed using RNA polymerase gene primers [14] as described previously [15]. For DNA sequencing the PCR reaction was scaled up to 100 μ l using antibody inactivated Taq

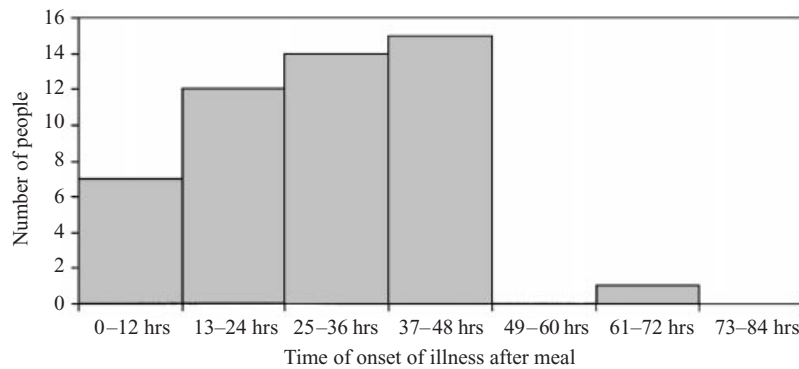


Fig. 1. Time of onset of illness of individuals involved in this outbreak.

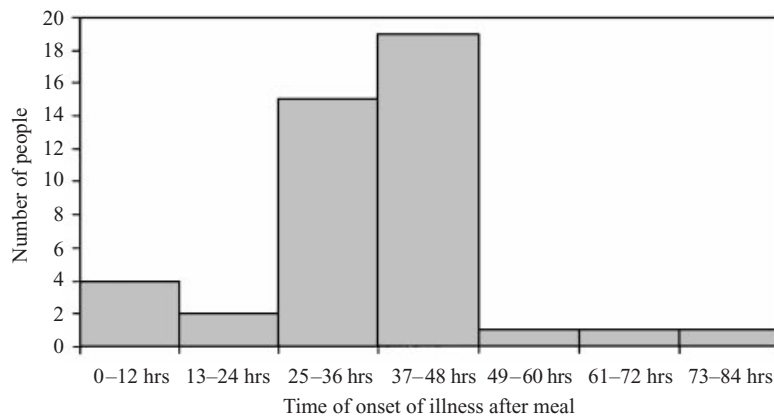


Fig. 2. Time of onset of diarrhoea and/or vomiting of individuals involved in this outbreak.

Table 1. Summary of characteristics of illness of 52 guests reporting to be ill in this outbreak

Symptom	Number	Percentage
Nausea	29	58
Diarrhoea and vomiting	21	42
Vomiting (without diarrhoea)	11	21
Diarrhoea (without vomiting)	11	21
Abdominal pain	20	40
Fever	19	38

(2.5 U Platinum Taq/reaction, Life Technologies) and amplification for 40 cycles: 94 °C, 30 s (1st cycle 3 min); 50 °C, 30 s; 72 °C, 10 s (last cycle 7 min) and the PCR product purified by isopropanol precipitation or using QIAmp PCR product purification columns (Qiagen Ltd). Direct sequencing of the 155 bp polymerase gene amplicon was performed using the Applied Biosystems 377 automated sequencer (Department of Pathology and Microbiology, Bristol University). PCR products were sequenced in both strands using the same primers as for amplification.

RESULTS

Epidemiological investigation

Eighty-three of the 126 guests returned completed questionnaires (response rate 66%). Fifty-two of the 83 responders reported illness (attack rate of 63%), of whom 43 (83%) suffered from either diarrhoea or vomiting or both.

The time of onset of symptoms was established for 49 of the diners. Eighty-four percent of those who became ill did so between 13 and 48 h after the meal and 59% between 25 and 48 h (see Fig. 1). Thirty-four of the 43 people (79%) reporting diarrhoea or vomiting or both had an onset of their diarrhoea or vomiting between 25 and 48 h (see Fig. 2). Of the 14 people who reported precise times for the onset of their illness the mean time from exposure to onset of symptoms was 33 h and the median 35 h. Details of the symptoms suffered were available for 50 diners and these are summarized in Table 1. From the questionnaires relative risks were calculated for the various foods consumed during the meal. None of the foods served was significantly associated with illness.

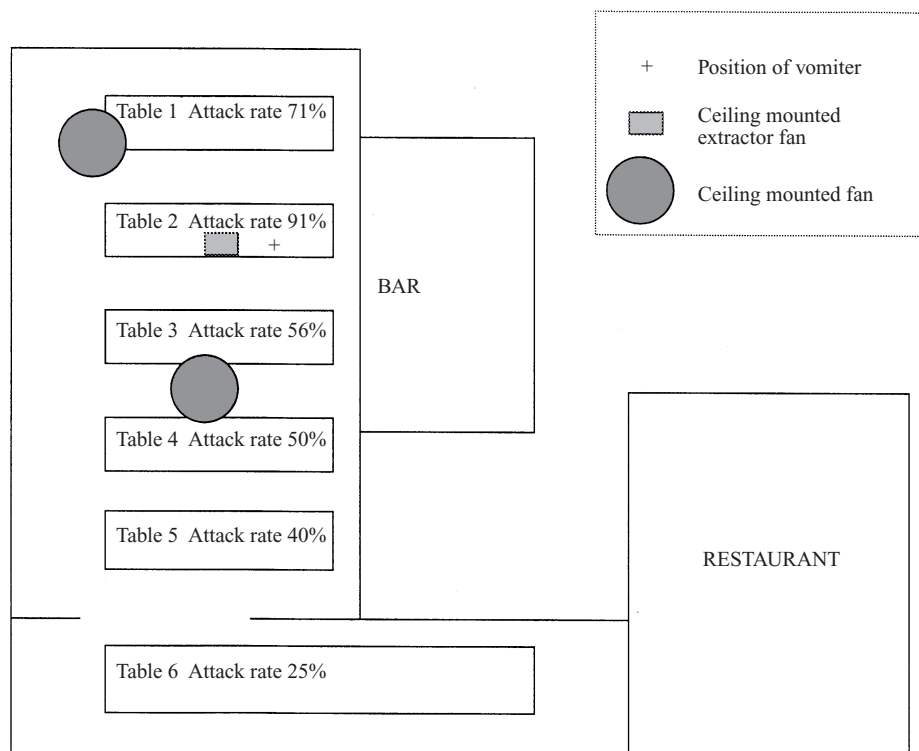


Fig. 3. Plan of the layout of tables in the restaurant. The locations of the index case and those who subsequently became ill are indicated.

Table 2. Summary of results of electron microscopy and RT-PCR on stool samples

Table no.	Total number of stool specimens submitted	Number of stool specimens positive for NLV on electron microscopy	Number of stool specimens positive for NLV on PCR	Number of stool specimens negative for NLV
1	1	0	1	0
2	0	0	0	0
3	4	1	2	2
4	2	0	1	1
5	1	0	0	1
6	1	0	1	0

The layout of the dining room and the arrangement of tables was as shown in Figure 3. Figure 3 also shows the positions of two ceiling mounted fans and an extractor fan which was located in the ceiling over table 2, almost directly above the vomiter.

Attack rates were calculated for the different tables at which the parties were seated (Fig. 3). The lady who vomited was seated at table 2, which had the highest attack rate, with the attack rate decreasing with spatial distance from her. Table 6 was situated through a large archway in a separate room from tables 1–5. A χ^2 test was performed using the numbers of people who became ill or who were not ill on each

of tables 2–6. The χ^2 value was 12.97 with 4 D.F. ($P = 0.01$). χ^2 for linear trend with 1 D.F. was 11.47 ($P = 0.0007$) and χ^2 for deviation from trend was 1.50 with 3 D.F. ($P = 0.68$). There is a highly significant relationship between distance from the vomiter and the risk of becoming ill with no significant deviation from that trend.

In addition to the party of 126 diners, the restaurant which is situated in a completely separate room was also occupied by hotel residents and casual diners. None of these subsequently became ill. One member of the waiting staff (not the one who cleaned up the vomit) developed a similar illness to the diners 24 h

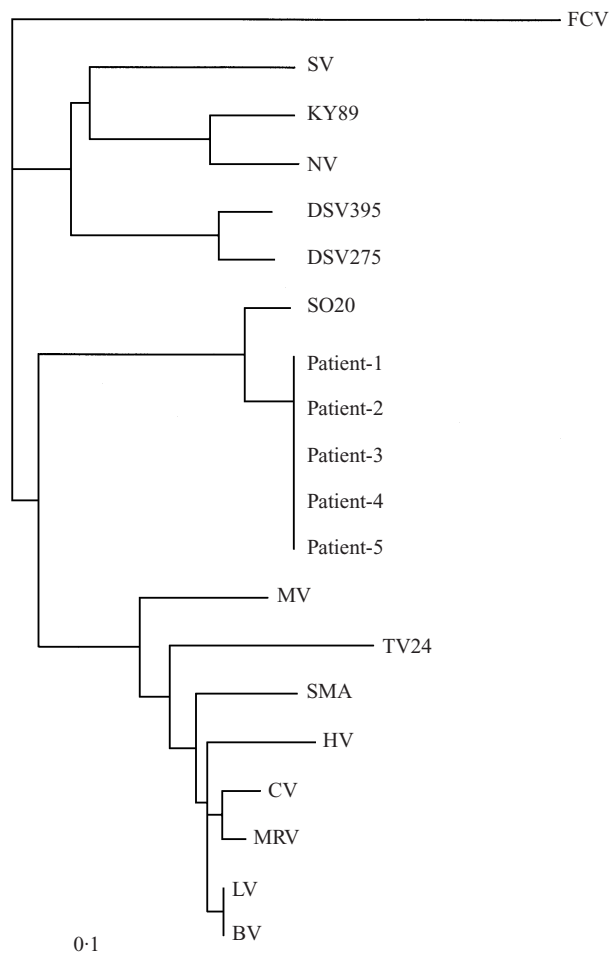


Fig. 4. Dendrogram showing the genetic relationships of the polymerase region amplicons of NLV strains identified among diners in this outbreak, with the equivalent region of characterized strains from the GenBank database. Accession numbers for strains include: SV, Southampton (L07418), KY89, KY-89/89/JPN (L23828); NV, Norwalk (M87661); DSV395, Desert Shield 395 (U04469); DSV275, Desert Shield 275 (U04538); SO20, S020/94/UK (Z73999); TV24, Toronto (U02030); MV, Melksham (X81879); SMA, Snow Mountain (L23831); HV, Hawaii (U07611); CV, Camberwell (U46500); MRV, Maryland (U07612); LV, Lordsdale (X86557); BV, Bristol (X76716). The length of the abscissa to the connecting node is proportional to genetic distance between sequences. The scale bar represents nucleotide substitutions per site.

after the vomiting episode. She recovered within 24 h. No members of the kitchen staff developed any symptoms.

Laboratory investigations

Only nine stool samples were returned and all of these were formed stools collected after the patients had recovered. All the stool samples were negative for

bacterial pathogens, but Norwalk-like viruses were seen by electron microscopy in one sample. This sample and four others were NLV positive by RT-PCR (see Table 2).

In all cases the RT-PCR primers produced amplicons with both Group I and Group II primers. The RT-PCR primers have to be broadly reactive, because of the highly variable sequence of NLV strains. As a consequence, genogroup discrimination may be lost, leading to the result obtained. An alternative explanation for these results would be a mixed infection with Group I and Group II viruses [15, 16] as mixed infections will also yield PCR products with both Group I and Group II primers. As a result, genotype information could not be assigned initially. In order to characterize the viruses involved in this outbreak, nucleotide sequencing was carried out with both group I and group II PCR products. A 96-nucleotide region was obtained and aligned against available NLV sequences. In all cases a single strain of NLV from genogroup II was identified with identical nucleotide sequence (Fig. 4). This sequence represented an unusual group II virus that did not cluster closely to any of the well characterized strains in the literature. Interrogation of the EMBL database of viral sequences (Release 58, March 1999) revealed very similar NLV sequences (98% identity) from outbreaks in Hokkaido Japan (accession number AB019261). The closest UK strain designated S020 (accession number Z73999) from an outbreak reported in Southampton in 1994 showed 93.75% nucleotide sequence identity [17].

DISCUSSION

All of Kaplan's Criteria [18] for the diagnosis of gastrointestinal outbreaks likely to be associated with NLV were met by this outbreak, namely:

- Stools negative for bacterial pathogens.
- A mean or median duration of illness of 12–60 h.
- A mean or median incubation period of 24–48 h.
- Vomiting in at least 50% of cases.

Hedberg and Osterholm [7] have proposed that having more cases with vomiting than fever in an outbreak could be used as a further epidemiological criterion for NLV outbreaks. In this outbreak 32 people suffered from vomiting and 19 had fever, i.e. Hedberg's criterion was met, further supporting the hypothesis that this outbreak was caused by NLV.

In addition to meeting Kaplan's and Hedberg's criteria, NLV was detected in the stools of patients by electron microscopy and PCR. Sequence analysis revealed a genogroup II NLV as the likely causative agent of the outbreak and the sequence was identical in all cases, consistent with a common point source of infection. The sequence type identified is unusual, showing only 69% identity with the closest well characterized strain (Melksham virus). In the United Kingdom, the most prevalent strains in circulation are Lordsdale-like viruses [13] although a similar variant to the one identified in this outbreak has been reported previously in the United Kingdom [17].

From univariate analysis no food served at the dinner could be implicated as the vehicle for transmission of the virus. A clear dose response pattern with distance from the vomiter and the fact that no one from the separate restaurant was subsequently ill suggest that an airborne route of transmission is the most plausible explanation for this outbreak. Only one member of staff out of the 12 who were serving at the tables became ill. This is a much lower attack rate than experienced by the diners. It is possible that airborne transmission to the waiting staff was reduced due to the intermittent nature of their occupation of the dining room. The diners however would be exposed continuously to the atmosphere in the dining room. However, it remains possible that aerosolization of virus particles led to contamination of food and/or hands and subsequent ingestion of the virus.

Unfortunately, no sample was obtained from the index case in this outbreak to confirm the sequence of the strain. However, the fact that all diners were infected with the same virus type and the clear epidemiological and statistical data showing that the attack rate of infection was directly proportional to distance from the index case, strongly implicate the vomiting incident as the source of infection. In spite of the fact that the vomiting incident during the meal was not particularly explosive or projectile, this outbreak clearly illustrates the risk to other diners posed by a subject vomiting in the same room, even when they are seated some distance away. In this incident the presence of ceiling fans may have contributed to spread of the virus.

The outbreak described here is consistent with airborne transmission of NLV from a subject who vomited during the meal, leading to inhalation and subsequent ingestion of the virus by other persons in the same room. It suggests that any vomiting can

potentially generate aerosols of virus and that these can pose a significant risk of transmission of the virus.

Inadequate cleansing of surfaces, particularly food preparation surfaces following vomiting, could lead to further cases, a pattern often seen in nursing and residential homes. Airborne transmission may represent a significant risk, not only in hotel and restaurant situations, but also in hospital wards and nursing homes. Appropriate infection control measures to deal with airborne transmission of virus particles [8] must be considered in these situations.

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