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A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination

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SUMMARY

An outbreak of Norwalk-like calicivirus (NLV) gastroenteritis occurred in a rehabilitation centre in southern Finland between December 1999 and February 2000. An epidemiological investigation was conducted to determine the source and extent of the outbreak. More than 300 guests and staff members became ill during the outbreak. No food or activity in the centre could be linked epidemiologically to illness. NLV genogroup II was detected by RT–PCR in stool samples of symptomatic guests and employees. All strains reacted similarly with the microplate hybridization probe panel and showed the same nucleotide sequence, indicating that they represented the same NLV strain. Food and water samples were negative for NLV, whereas NLV was detected in three environmental specimens. The strains from patients and environment were identical based on microplate hybridization probes, suggesting that environmental contamination may have been important for the spread of calicivirus and the protracted course of the outbreak.

INTRODUCTION

The Norwalk and Norwalk-like caliciviruses (NLV) are the most common aetiological agents of epidemic gastroenteritis in adults. The role of NLV as a frequent cause of gastroenteritis was established in the 1970s and 1980s [1, 2]. The virus is mainly transmitted from person-to-person through the faecal-oral route, but can also spread through ingestion of contaminated food or water, or by airborne droplets created during vomiting [3, 4]. Food and drinking water are common sources of NLV outbreaks, and swimming in contaminated water has also caused outbreaks [5, 6]. Nursing homes and hospitals are common settings for outbreaks [7]. During 1990–5, outbreaks in these institutions accounted for 76% of the total of NLV gastroenteritis outbreaks in England [8]. Persisting outbreaks have been reported in hotels and on cruise ships [9, 10].

Diagnostic methods for calicivirus infection have improved substantially during the 1990s. Reverse transcriptase polymerase chain reaction (RT–PCR) assays have proved to be sensitive for clinical diagnosis and are replacing the less sensitive electron microscopy, which is unable to distinguish between different small round structured viruses (SRSV). RT–PCR has been successfully used for viral detection in environmental samples such as water [11, 12], oysters [13], and delicatessen sandwiches [14]. Se-

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quence analysis of the PCR amplification products of the NLV polymerase (ORF1) and/or the capsid (ORF2) region from patient and environmental samples has enabled tracing the source of an outbreak [11, 15].

Between December 1999 and January 2000, a prolonged NLV outbreak occurred in a rehabilitation centre in southern Finland. We conducted epidemiological, environmental and virological investigations to determine the source and mode of transmission of the outbreak.

METHODS

Setting and outbreak

Rehabilitation centre A had 180 double rooms located in two different buildings, each having two sections for accommodation. There were more than 200 employees, including health care workers, administrative, kitchen, cleaning, and maintenance staff. The centre had facilities for treatments and activities, including physiotherapy, massage, herb-baths, foot therapy, motion therapy in pools, and gym rooms. Drinking water was non-chlorinated ground well water from the municipal water supply. Meals served in the restaurant were prepared in the facility's own kitchen. Most guests were elderly people, who usually stayed for treatment 1–3 weeks.

On 30 December 1999, the National Public Health Institute (KTL) was notified that many guests had vomiting, diarrhoea, and fever in the rehabilitation centre A. The first patients became ill on 21 December and during the next 10 days more than 50 persons contacted nurses of the centre because of gastroenteritis. After New Year the number of cases decreased without any control measures. However, on 25 January 2000, KTL was informed about a sudden increase of new cases with gastroenteritis with onset within the last 24 h among guests and staff of the centre.

Epidemiological investigation

The occupational health nurses of the rehabilitation centre collected information on the number of guests and staff who contacted them because of gastrointestinal symptoms. To determine the source of the outbreak, data were collected on all guests ($n = 280$) who stayed in the centre from 24 December to 26 December by using a postal standard questionnaire. At the end of January, all 20 employees of a pharmacy, who stayed in the centre from 22 January to 23 January were interviewed face-to-face by using a standard questionnaire. In both studies, participants were asked about activities and treatments in the centre, meals and food items eaten during the stay, and time and symptoms of subsequent gastroenteritis. A case was defined as a visitor to the centre between 24 and 26 December or between 22 and 23 January, who had acute gastroenteritis (diarrhoea and/or vomiting and/or nausea) with onset between 25 and 29 December, or between 23 and 27 January, respectively.

The data were analysed by using Epi-Info version 6.04 (Centres for Disease Control and Prevention [CDC], Atlanta). Food-specific and activity-specific attack rates and relative risks with 95% confidence intervals were calculated.

Environmental investigation

All treatment areas, saunas, swimming pools and kitchen facilities as well as sections where guests were accommodated were inspected on 27 January 2000. The water supply system was assessed by the local environmental health unit in collaboration with the Division of Environmental Health of KTL.

Laboratory investigation

Faecal samples for microbiological analysis

Sixty-seven persons provided 83 stool samples for virological examination. Twenty-eight specimens were from symptomatic guests and 55 samples from kitchen staff. Sixteen samples from staff members were taken at the end of December, and 39 samples at the end of January; second samples were provided from the same 16 employees and first samples from 23 other staff members.

At the end of December, 12 stool samples from symptomatic guests and 16 samples from staff members were also cultured for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas* and *Plesiomonas* species. Nine samples obtained at the end of January were cultured for the same bacteria.

Environmental samples for virological analyses

Water samples from the water supply system were collected on 4 January 2000. On 18 January water

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samples were taken from all four swimming pools of the centre. On 27 January, 30 swab samples were taken from the surfaces of 2 accommodation rooms with symptomatic guests, 2 sauna rooms, 2 bath rooms, 2 gym rooms, the ultrasound treatment room, the main entrance and the restaurant. Surfaces were brushed with either wet or dry cotton swabs, which were put into a tube containing 0·5 ml phosphate buffered saline (PBS) and stored at 4 °C.

Virological methods

Water filtration was performed as previously described [16]. Briefly, one litre of water was filtered through a positively charged membrane, eluted with glycine-beef extract pH 9·5, and further concentrated with microconcentrator to a final volume of 100 μl. Nucleic acids were extracted with Tripure reagent from 10% stool suspensions in 0·05 M Tris–HCl/0·1 M NaCl, 1 mM $CaCl_2$, pH 7·4, water filtration product and environmental swab samples. NLV-RT–PCR detection for genogroups I and II and confirmation with microplate hybridization was performed and nucleotide sequences of three NLV amplicons were determined as previously described [16]. The first five patient samples were also examined by electron microscopy and astrovirus-RT–PCR with primers of Mitchell et al. [17].

Control measures

On 27 January infection control measures were implemented. Accommodation rooms were cleaned and disinfected one section at a time, and new guests were accommodated in the cleaned sections. All surfaces in bathrooms and environmental surfaces in the rooms were disinfected with 500 ppm hypochlorite solution. Physiotherapy rooms and instruments were disinfected daily with 500 ppm hypochlorite. The chlorine concentration of swimming pools was increased from 1·0 to 2·5 mg/l each night. Alcoholic hand rub was made available in common toilets, treatment rooms and at the cafeteria. Guests were advised to pay special attention to hand hygiene. They were also instructed to refrain from treatments and to contact the nurses if gastrointestinal symptoms developed. After implementation of control measures, the number of cases decreased rapidly, and closure of the centre was not considered necessary.

RESULTS

Epidemiological investigation

The epidemic curve (Fig. 1) shows the first persons had gastrointestinal symptoms on 21 December and thereafter the outbreak occurred in three waves. The first was from 27 December to 5 January, the second one from 10 January to 20 January, and the third from 24 January to 5 February. Altogether 331 persons with symptomatic infection were recorded; 118 of them were employees and 213 were guests. Staff members with symptomatic illness were found in all professional groups. The first illness among kitchen staff was recorded on 27 December.

In the first cohort study, 208 persons completed the postal questionnaire (response rate 74%). One hundred and twenty-five (60%) met the case definition. None of the 29 food items eaten at meals between 24 December and 26 December was significantly associated with illness; the relative risks varied from 0·71 to 1·27.

Among members of pharmacy staff, 11 respondents met the case definition (AR 55%). All were women, and median age was 46 years (range 28–60 years). Symptoms began the next day after leaving the centre for most cases (7/11). The duration of illness was less than 3 days for all cases. None of the 39 food items or of the various activities conducted in the centre was significantly associated with illness.

Environmental investigation

The treatment facilities, saunas, swimming pools and kitchen were all clean and well maintained. No disinfectant was used for cleaning the equipment in the gym room. In physiotherapy and massage departments, the treatment tables were wiped with disinfectant after each client. All treatment rooms were daily cleaned, but disinfectant was not used systematically. Investigation of the water supply system did not reveal any sites with risk of contamination.

Laboratory investigation

Patient samples

Bacterial cultures of the stool samples taken from guests and from staff did not yield any enteric pathogens. NLV genogroup II (GII) was the only virus detected in the virological investigations (Table

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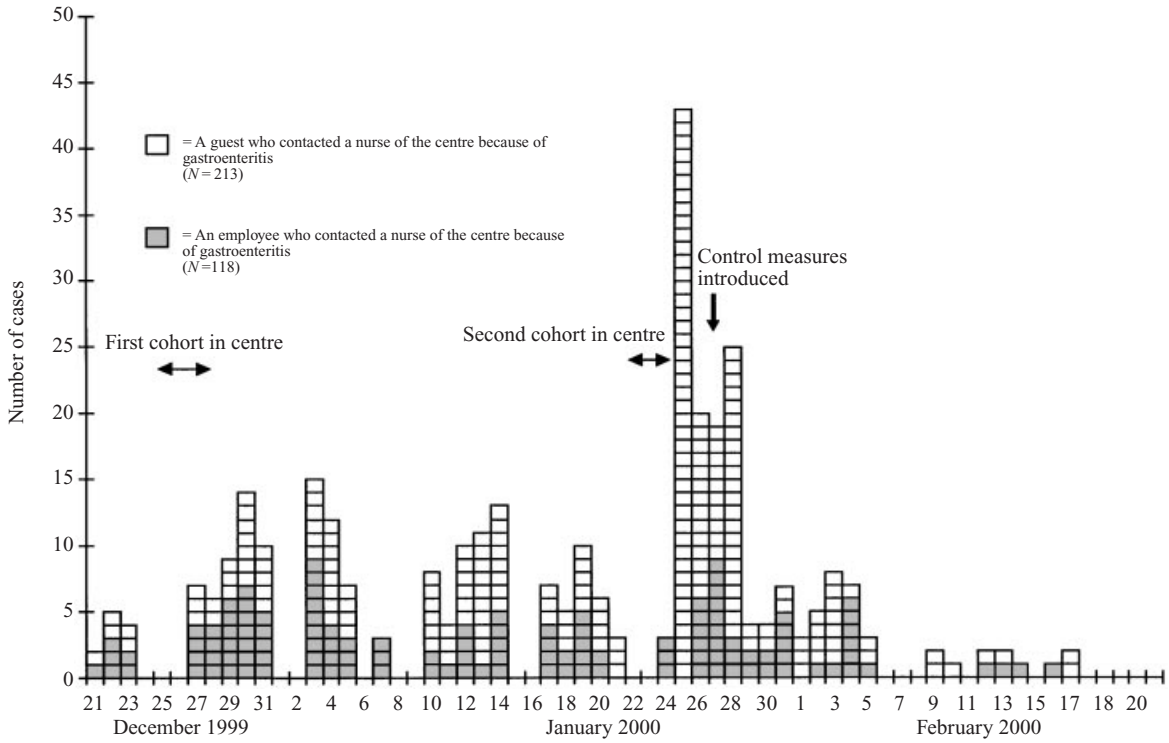

Fig. 1. Illness by recording date among guests and employees at rehabilitation centre A, 21 December 1999 to 21 February 2000.

Table 1. *NLV RT–PCR results during the outbreak in rehabilitation centre A*

	Number of NLV RT–PCR positive samples (%)			
Samples from	December 1999	January 2000	February 2000	Total
Guests	4/5 (80)	10/14 (71)	5/9 (56)	20/28 (71)
Kitchen staff	5/16 (31)*	3/16 (19)*	—	15/39 (38)
		7/23 (30)		

* Same staff investigated in December 1999 and January 2000.

1). The virus was detected in 4 of 5 samples from guests with illness with onset between 30 and 31 December, and in 5 of 16 samples from kitchen staff taken at the same time. Three samples from members the same group of 16 kitchen workers who were negative in December were positive for NLV GII in the end of January. Six of 10 samples from the pharmacy staff were positive in NLV–PCR. In total, 71% of samples from guests and 38% of samples from staff were positive for NLV GII.

All NLV positive PCR products reacted similarly with the microplate hybridization probe panel suggesting that they represented the same NLV virus strain. The identity was further confirmed by nucleotide sequence determination of three of the NLV amplicons. Nucleotide sequence of this NLV strain resembled that of the Hawaii strain with only three nucleotide differences in the 66-base region.

Environmental swab samples

NLV–PCR was positive in four swab samples (an ultrasound physiotherapy instrument's handle, a bathroom door handle in a room of a symptomatic guest, a toilet seat in a room of a symptomatic guest and a toilet seat in a public toilet for ladies. Based on the reactivity to the used microplate hybridization probes the environmental NLV strain was identical to the strain detected from patient samples. Sequencing of the environmental strain was not successful.

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Water samples

Tap water samples taken on 4 January were all negative for NLV. Water samples from swimming pools were also negative for calicivirus.

DISCUSSION

A prolonged gastroenteritis outbreak occurred among guests and staff in rehabilitation centre A. The attack rates among guests were high; more than half of respondents met the case definition. More than half of examined stool samples from guests and members of staff were positive for NLV calicivirus, and all the strains belonged to the same genogroup (GII) supporting the hypothesis that a single NLV strain caused the outbreak. Sequencing of the environmental strains was not successful due to the low concentration of amplicon obtained.

In epidemiological studies, no food or activity could be linked to the illness. In addition, no pathogens were found in investigated foods, tap or pool water samples. The first illness onset among kitchen staff occurred after the outbreak had already begun. We found no evidence that the outbreak was caused by a contaminated food arriving to the kitchen or by food contaminated by an infected kitchen worker.

More than half of the employees of the centre had symptomatic infection. Because not all calicivirus infections are symptomatic [18], the majority may have been infected in the course of the outbreak. Many employees had close contacts with the guests (e.g. during physiotherapy) facilitating the spread of the infection. We could not find a common source of the outbreak, but the epidemic curve is consistent with the hypothesis that after the virus was introduced to the centre, it was transmitted from person-to-person. This type of spread is typical in most calicivirus outbreaks [19–21], and probably was also important in this outbreak.

Since only a few caliciviruses are needed to cause infection, contaminated surfaces may be an important source of infection. NLV detected from environmental swabs have been reported in a hospital outbreak [19], and in a prolonged hotel outbreak [22]. In the latter study, toilet rims and seats, carpets, horizontal surfaces, toilet handles and taps, frequently handled objects and soft furnishings were positive for NLV calicivirus by RT–PCR-assays. On the other hand, in a prolonged outbreak in a long-term facility, environmental samples were RT–PCR negative [20]. Sampling methods may be important for detection of caliciviruses; in the long-term facility outbreak [20], dry cotton swabs were used. In our study, NLV was detected from both moistened and dry cotton swabs. Positive PCR results may also be obtained from non-viable virus. However, the NLV RNA-genome is susceptible to RNAses found widely in the environment, and the findings probably represent viable NLV viruses. Our results suggest that environmental contamination may have been important for the transmission of NLV and the prolonged course of the outbreak.

Calicivirus outbreaks can be prolonged in settings where new cohorts of susceptible people are introduced [9]. The beginning of each of the three outbreak waves coincided with arrival of a large number of new guests to the centre. The outbreak continued for nearly 2 months, and the highest attack rate occurred about 1 month after the outbreak began. In an epidemiological study of non-bacterial gastroenteritis outbreaks reported to Centers for Disease Control, a NLV-outbreak in a hotel with 3000 guests persisted for 34 days [23]. In a previous study, the mean duration of SRSV outbreaks in hotels and restaurants was 6 days, and one outbreak lasted for 23 days [9], and in another study the median duration for outbreaks in long-term care facilities was 9 days [8]. A rehabilitation centre may be an especially hazardous setting for calicivirus outbreaks, because most guests are elderly people, and new susceptible people arrive continuously in the centre.

This outbreak ended rapidly after implementation of control measures. Cohorting of healthy newcomers to cleaned and disinfected sections may help to control calicivirus outbreaks in institutions. On the other hand, control measures were implemented rather late during the outbreak, when most employees were already infected. It is also possible that the main reason for controlling the outbreak was that most susceptible members of staff had already been infected and ceased to transmit the virus.

Calicivirus outbreaks are difficult to control because the virus is spread easily from person-to-person. Our results suggest that environmental contamination may also be important for the transmission of calicivirus, and therefore disinfection of contaminated surfaces and adequate hand washing practices should be promptly introduced to stop transmission. Symptomatic persons should avoid close contact with other people before they have been asymptomatic for at least 48 h.

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