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Investigation of an outbreak of gastroenteritis caused by Norwalk-like virus, using solid phase immune electron microscopy

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Summary: In February 1993, 95 persons (47 patients and 48 staff members) were affected by an hospital outbreak of viral gastroenteritis. Using direct electron microscopy (EM) the causative agent was identified as a small round structured virus. This was confirmed as a Norwalk-like virus using solid phase immune electron microscopy (SPIEM). Of 94 stool samples examined, 12 (13%) samples containing small round structured viruses (SRSV) were SPIEM positive for Norwalk-like virus. A further 25 (27%) samples contained small round featureless virus (SRFV) identified by direct EM and were negative on SPIEM. The illness was characterized by preceding influenza-like symptoms in 76% of cases followed by vomiting (76%), diarrhoea (79%) and abdominal pain (79%). One fatality was recorded. The outbreak lasted for 15 days, with a peak incidence of new cases amongst patients and staff occurring on day 5. It was controlled through a combination of ward closures, patient cohorting, suspension of duties for affected staff and disinfection procedures. Difficulties were encountered in the education of staff and in the implementation of environmental control measures. Screening of hospital catering services and a case control study, carried out among affected staff members, failed to identify a foodborne source. Consumption of tap water in the hospital was commoner among affected staff members than among controls, but this did not reach significance ($P = 0{\cdot}1$).

Keywords: Norwalk virus, small round virus, immune electron microscopy, outbreak.

Introduction

Norwalk virus is a small round structured virus (SRSV) recently classified as a Calicivirus, distinct from small round featureless viruses (SRFV) such as Enteroviruses and Parvoviruses. It was first identified in 1972 as the cause of Winter Vomiting Disease. Since then it has been increasingly recognised as a common cause of institutional outbreaks of gastroenteritis. Infection is commonest at the extremes of age. The characteristic features of outbreaks of Norwalk virus gastroenteritis were described by Kaplan[1] and are shown in Table I. Transmission is primarily fecal-oral although other routes such as environmental contamination by infected vomitus[2] and direct airborne person to person spread[3] may be involved leading to a high secondary attack rate. Outbreaks may be linked to water consumption or recreational water use.[4] Foodborne outbreaks may be due to primary sources such as shellfish but are more usually due to

Table I *Characteristic features of outbreaks of norwalk virus gastroenteritis*[1]

- Routine stool cultures negative
- Mean duration of illness 12 to 60 hours
- Vomiting in >50% of cases
- Incubation period of 24 to 48 hours

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secondary contamination of food by symptomatic food handlers.[5] This paper describes the investigation and management of a hospital outbreak of viral gastroenteritis.

Methods

Microbiological methods

Solid phase immune electron microscopy (SPIEM) is a method of serologically trapping viral particles on to electron microscopy (EM) grids. High titre convalescent serum from previously infected patients is bound by anti-human IgG to carbon/formvar coated copper grids. This, in turn, binds to viral particles in the stool specimens trapping the virus on the grid and allowing specific identification of these particles when negatively stained and viewed by EM.

As the method relies on the capture of virus particles directly from clarified suspensions of human faeces on to electron microscopy grids coated with diluted convalescent serum, the choice of a suitable convalescent serum is vital to the success of the technique. Late convalescent sera from previous U.K. outbreaks of serotypes 2 and 3 were obtained from Bristol Public Health Laboratories (Caul & Ashley) for use in our work.

Formvar/carbon coated EM grids were coated with a 1:500 dilution of anti-human IgG by incubating the grid on a drop of serum for 1 hour at room temperature in a moist chamber. The grids were then coated with capturing antibody by incubating the grid on to a drop of diluted, previously standardized, human convalescent serum. Faecal emulsions were prepared as 5% dilutions in minimal essential maintenance medium. The emulsions were clarified by centrifugation at 1500 ***g*** for 20 minutes. The coated grids were then incubated on top of a drop of clarified faecal emulsion for two hours at room temperature.

Grids were examined by EM at an instrument magnification of 50 000. Four grid holes were examined before calling any specimen negative. Because of the strong washing procedure in the final stage of the SPIEM protocol, very clean images were viewed by EM with very little or no cellular or bacterial debris remaining on the grids. Positive and negative controls were run with all SPIEM runs.

Faecal specimens were cultured for viruses by inoculation into fetal rhesus kidney, HeLa and Vero cell lines, and incubated at 37°C for 10 days.

Epidemiological methods

A case control study was carried out, confined to staff members as there was difficulty in obtaining information from patients given the population involved. A detailed questionnaire was completed by 29 symptomatic staff members and 19 controls matched for age, sex and work area. This included details of symptoms and food (both hospital and non hospital) consumed in the 72 hours prior to the onset of symptoms or to a matched date in controls. The case definition used in epidemiological investigations was any patient or staff member with otherwise unexplained vomiting or diarrhoea and one or more of the following symptoms: nausea, abdominal pain, headache or a preceding influenza-like illness.

Analysis of data was carried out using EPIinfo software (CDC, Atlanta). Categorical data were analysed using the χ^2 test.

Outbreak description

On day one of the outbreak, three patients and three staff members on a mixed geriatric and general medical ward became ill with vomiting and diarrhoea, associated with an influenza-like prodrome. From day two to four, 29 new cases were reported in the geriatric and neighbouring wards. The number of new cases increased on adjacent wards, peaking on day five. A smaller peak occurred between days 10 to 15 involving staff and patients in a different area of the hospital. This was associated with affected medical staff returning to work prior to 48 hours after the resolution of their symptoms. The outbreak lasted for 15 days and, in all, 95 patients and staff fulfilled the case definition. The outbreak curve is shown in Figure 1. Of 47 affected patients (19 on the geriatric ward) there was one associated death, an 84-year-old male with underlying carcinoma. There were 48 staff members affected (23 working on, or associated with, the geriatric ward). This included seven catering staff. The frequency of reported symptoms in the 29 staff surveyed as part of the case control study were: vomiting 22(76%), nausea 11(38%), abdominal pain 23(79%), diarrhoea 23(79%), headache 6(21%) and a preceding influenza-like illness 22(76%).

Results

Identification of pathogen

The initial symptomatology was thought to be typical of viral gastroenteritis. This was confirmed by

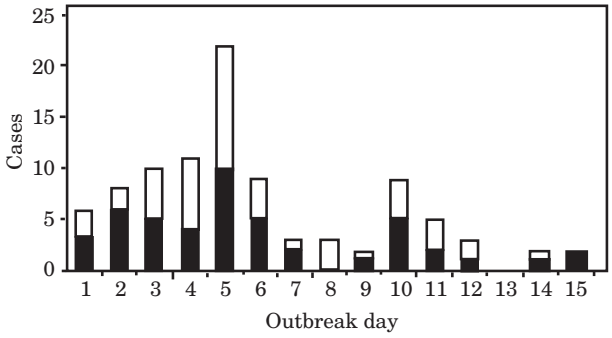

Figure 1 Number of new cases presenting each day from 8/2/93 to 23/2/93 □, staff; ■, patients.

standard EM on day two of the outbreak with a SRSV identified in four of the initial specimens. Based on morphological findings this SRSV was presumptively identified as a Norwalk-like virus. Of 94 faecal specimens examined using standard EM, 12(13%) were found to contain SRSV and 25(27%) contained a homogeneous group of SRFV (24–32 nm diameter). SPIEM was carried out on the 37 specimens in which viruses were detected on standard EM. SPIEM was positive for Norwalk-like virus on all 12 of the specimens containing SRSV and negative on all 25 specimens containing SRFV. Viral culture, carried out on 12 of the specimens containing SRFV, was negative.

Control measures

An emergency infection control committee, chaired by the consultant microbiologist, was convened and an initial press statement prepared. Case identification was commenced using the case definition. Affected patients were cohorted and admission to and transfers from the geriatric ward were stopped. Strict enteric precautions were put in place and a 70% alcohol hand rub was introduced to supplement routine hand washing procedures. Affected staff were sent home and advised to stay off work until 48 hours after symptoms subsided, as Norwalk virus has been shown to be potentially transmissible up to this time. Decontamination procedures were changed from standard phenolic solution to a 2% hypochlorite solution as recommended by Breuer and Jeffries.[6] Difficulties were encountered in implementing this change as the solution was corrosive and in particular was found to corrode commode seats on the ward.

Epidemiological investigations

Catering inspections were carried out and no breakdown in standard hygiene procedures were found. Food and water samples were sent for culture but no virus, or bacterial faecal indicator organisms, were detected. Two members of catering staff were found to be symptomatic at the beginning of the outbreak, one of whom was involved in serving food in the geriatric ward in the 48 hours prior to the outbreak.

The case control study failed to identify a common food source. However there was an association with drinking water from the hospital water supply (16 symptomatic vs. 6 non-symptomatic), though this did not reach statistical significance ($P = 0{\cdot}1$). A review of community care records showed no increase in the reported rate of gastroenteritis and faecal indicator bacteria were not detected in the local water supply at that time. Water-borne outbreaks have been reported to occur following heavy rainfall[7] but review of meteorological records showed a low level of rainfall in the Dublin area for the period in question.

One staff member developed the illness, with positive SPIEM, without direct contact with patients or staff in the affected wards and without a significant food history. The only risk factor identified in this case was walking through the geriatric ward.

Discussion

There were a number of potential sources for this outbreak. A primary foodborne source is unlikely, but secondary food contamination from a symptomatic food handler may have been involved in the initial patient cases in the geriatric ward. A water-borne source was suggested from the case control study and is not ruled out by the failure to detect virus or faecal indicator organisms in water samples at that time. Although faecal indicator organisms are a reliable measure of faecal contamination, SRSV outbreaks have been reported from water supplies in the absence of such organisms.[8]

The shape of the epidemic curve is typical of an outbreak with a high secondary attack rate caused by direct person to person spread. Although the transmission of SRSVs was initially shown to be faecal–oral in volunteer studies[9] recent work has suggested that infected vomitus is a more important source in the dissemination of the organism in

outbreaks.[10] Caul has estimated that, given a concentration in vomitus of 10^6 viral particles ml^{-1}, a single episode of vomiting would be expected to distribute 30 000 000 viral particles.[11] The importance of environmental contamination by infected vomitus is underlined by the report of recurrent outbreaks on a cruise ship, linked to this source.[12]

The transmission from infected vomitus is thought to be primarily via hand contamination[11] but probable direct airborne transmission has been described[3] though this remains an unproven route. Airborne transmission appears to have occurred in this outbreak, suggested by the affected staff member whose only exposure was walking through the geriatric ward.

The high secondary attack rate makes it difficult to identify the primary source. This is further compounded by the normal seasonal increase in viral gastroenteritis in the community at this time of the year. It is possible that this outbreak may have been part of a wider community outbreak but this seems unlikely as the level of reports of viral gastro-enteritis was no higher than for the same period in previous years.

The secondary peak occurring later in the outbreak was thought to be due to affected staff members returning to work less than 48 hours after symptoms had subsided. This underlines the importance of educating staff members about the importance of control measures. The high attack rate amongst staff members in Norwalk virus outbreaks is typical and extra staff members should be made available early in the course of the outbreak, to minimise the risk of affected staff being pressurized to return to work.

Viruses are not inactivated by phenolic disinfectants normally used for ward decontamination.[13] Although other viral causes of gastro-enteritis are inactivated by a 0·1% hypochlorite solution, SRSVs require a 2% solution.[6] We found this solution to be quite corrosive, particularly when used on some types of plastic. These difficulties underline the importance of education of domestic and ward staff prior to using such a solution.

The high percentage of SRFVs (27%) identified, despite negative viral stool cultures, is worthy of note. Although similar viruses have been reported from several outbreaks they are not generally considered to be causative agents of viral gastroenteritis.[14] However the striking numbers found in this outbreak raise questions regarding their significance, particularly as they were of an homogeneous morphology. The finding of such viral particles in association with Norwalk virus has previously been noted.[15] They may be associated viruses acting as a marker of recent gastrointestinal mucosal damage. Defective parvoviruses, which require co-infection with adenovirus for their complete replication, are known to occur in human infection.[16] Oliver and Phillips reviewed stools from children with acute diarrhoea in which SRFVs were identified.[17] They found that 75% of SRFVs were parvovirus-like and that half of these were associated with the presence of adenovirus particles. It is possible that SRFVs, such as those seen in this outbreak, may represent such a co-pathogen. Alternatively they may represent a true second pathogen, though this seems less likely. Further work is necessary to elucidate the true significance of these viruses. We intend to carry out a polymersase chain reaction (PCR) based study of SRFVs from this and other outbreaks in the future.

The identification of SRSVs by EM requires that specimens be taken within the first 48 hours of the illness, when the excretion of viral particles is at its highest. Even at this time the positivity rate is frequently very low as the concentration of these viral particles in faeces is usually 10^5–10^7 per gram, compared to 10^{10}–10^{12} per gram for Rota virus gastroenteritis.[5] SPIEM has previously been shown to give a nine-fold increase in the sensitivity of Rota virus detection in stools when compared to standard EM.[18] The technique proved to be a valuable tool in confirming the identity of the pathogen in this outbreak. SPIEM is now used routinely at the Virus Reference Laboratory for investigation of potential outbreaks involving SRSVs and has been successfully employed in subsequent outbreaks from other institutions.

SPIEM can also be used to demonstrate a serological response to the outbreak virus, though this is an extremely labour-intensive procedure.[19] It has also been used to classify Norwalk-like viruses into different antigenic groups.[20,21] However SPIEM techniques are dependent on the availability of suitable convalescent serum and requires an experienced operator. A number of less labour-intensive diagnostic methods have recently been developed for SRSVs, including Norwalk virus. These include enzyme-linked immunoassays (EIAs) and PCR-based techniques. EIAs have been developed, based on baculovirus-expressed viral capsid proteins and hyperimmune antisera.[22] In addition to diagnosis of SRSV outbreaks,[23] these assays have provided better

insights into the natural history of Norwalk virus infection.[24] EIAs have also allowed for large scale sero-epidemiological studies of SRSV immunity.[25]

PCR has been shown to be a powerful tool in the diagnosis and characterisation of viral gastroenteritis. Astrovirus was detected in four of 16 (25%) stools from an outbreak among military recruits using reverse transcriptase-PCR (RT-PCR). Astrovirus was only detected in 2(13%) by EM[19]. RT-PCR was positive in 40% of specimens from a Norwalk virus outbreak on a cruise ship, compared to 29% detection by EM. In the same outbreak RT-PCR was used to characterise the genetic variation between the outbreak strain and reference strains of Norwalk virus.[26] The increasing availability of sensitive and specific diagnostic methods, such as EIA and PCR, should allow earlier detection and more effective control of SRSV outbreaks in the future.

Conclusion

Ease of transmission and high secondary attack rates make control of hospital outbreaks of SRSVs challenging. Attention needs to be paid to the role of symptomatic staff in propagating such outbreaks. Logistical difficulties may be encountered in implementing current recommendations on environmental inactivation of these pathogens. SPIEM is of value in confirming the identity and serotype of the outbreak strain. It is likely to be too cumbersome for initial identification of SRSVs in large outbreaks. In smaller outbreaks and individual cases, or where faecal specimens are not sent within 48 hours of the onset of symptoms, the potential increase in sensitivity over standard EM should increase the likelihood of detecting SRSVs. The role of SRFVs in viral gastroenteritis requires further investigation.

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