

# VALUE OF A REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION ASSAY FOR NORWALK-LIKE VIRUSES IN THE INVESTIGATION OF INSTITUTIONAL GASTROENTERITIS OUTBREAKS

*Mark J. Ferson, Peter Yankos, Giuletta Pontivivo, Angela Wong, David Lee*  
South Eastern Sydney Public Health Unit  
South Eastern Sydney Area Health Service

*Monica Isaacs, Fifin Intan, Christopher J. McIver, William D. Rawlinson*  
Virology Division and Enteric Laboratories  
South East Area Laboratory Services  
South Eastern Sydney Area Health Service

This article reports two outbreaks of gastroenteritis which occurred in nursing homes in South Eastern Sydney during November 1998 in which an assay, developed by the South Eastern Area Laboratory Services (SEALS) Enteric and Virology Laboratories, was used to confirm the aetiological agent as Norwalk-like viruses (NLVs). This investigation was done as part of the Public Health Unit's response to the notification of gastroenteritis in an institution.

NLVs, types of small round-structured viruses (SRSVs), have been recognised since the 1970s as being responsible for epidemic gastroenteritis. Outbreaks, generally affecting older children and adults, have been associated with contaminated food (especially oysters),<sup>1,2</sup> or water,<sup>3</sup> and with person-to-person spread in closed institutions such as nursing homes and hospital wards. Such outbreaks tend to have common characteristics, including an incubation period of 24 to 48 hours, explosive onset, preponderance of vomiting in affected persons, and a relatively brief duration of illness in the range of 12 to 60 hours. In these outbreaks, the number of people affected has varied from fewer than 10 to several thousand.

In NSW, institutional outbreaks of gastroenteritis are notifiable by doctors in accordance with the *NSW Public Health Act 1991*. Once doctors or hospital staff report an outbreak it is the responsibility of Public Health Units to work with the staff and management of the institution to control the outbreak. In general, due to the lack of availability of rapid testing for NLV, Public Health Units have only rarely sought testing for this group of agents.

In South Eastern Sydney Area Health Service, the South Eastern Sydney Public Health Unit and the SEALS Enteric and Virology Laboratories have collaborated over a number of years in investigating gastroenteritis epidemics in community settings. During 1998 SEALS Virology developed and introduced a reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detecting NLVs in stool samples that was suited to investigating outbreaks of acute gastroenteritis.

## METHODS

### Epidemiological and food investigation

On notification of a presumed outbreak of gastroenteritis in a community-based institution, the Public Health Unit mounted a public health response within 24 hours. This included an epidemiological investigation to ascertain the number and characteristics of people affected by the outbreak (cases), the severity and duration of illness, and any food which might be implicated as the source of infection. If the pattern of the outbreak or information provided by staff or cases suggested a point source (for example, contaminated food), a food investigation was also undertaken. Staff at the institution and the doctors caring for cases were asked to collect stool samples from cases early in their illness. These samples were delivered to the SEALS Enteric Laboratory, either by Public Health Unit staff or via a private pathology service.

### Microbiological methods

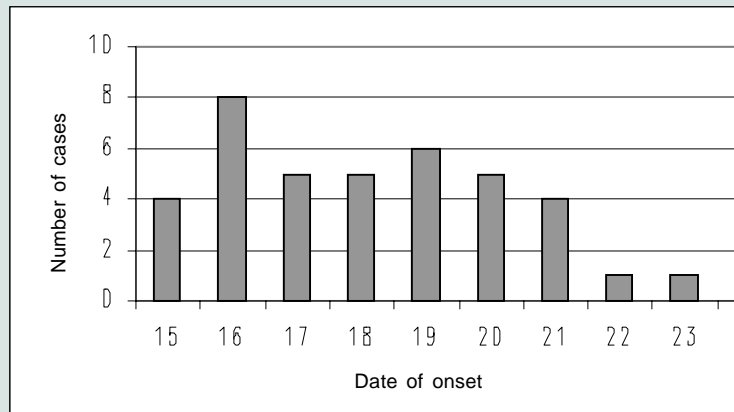
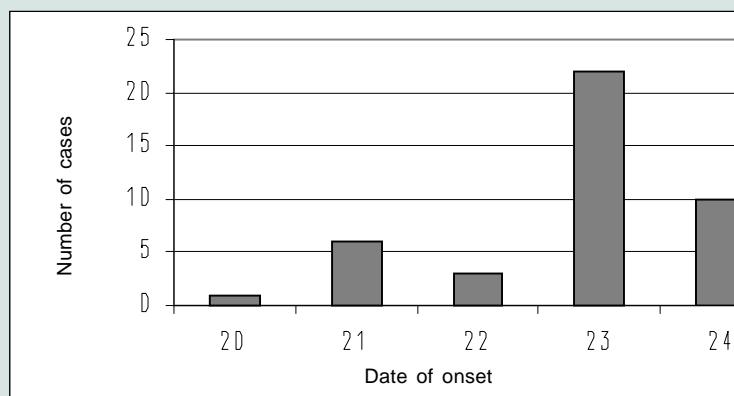
Unconcentrated faecal samples stained with iodine were examined for white blood cells, cysts, ova, and parasites, then were cultured for bacterial pathogens (salmonella, shigella and campylobacter) using standard techniques. Faecal antigens of rotavirus, adenovirus and astrovirus were tested using commercial enzyme immunoassays (DAKO, Australia). NLVs were detected using RT-PCR with primers derived from the nucleotide sequences of the Camberwell and Bristol viruses (SRSV genogroup IIb).<sup>4-6</sup>

## RESULTS

### Outbreak 1

On 23 November, a nursing home in Sydney's southern suburbs reported an outbreak of gastroenteritis affecting staff and residents that had begun on 13 November. Of 53 residents, 28 had been unwell with diarrhoea and vomiting lasting up to 72 hours (mean 44 hours). Ten members of the nursing staff had a similar illness, and cases ranged in age from 30 to 97 years. The last new case occurred on 24 November (Figure 4). Analysis of questionnaires showed no association with specific foods.

An inspection of the kitchens was carried out and a number of problems were found with food storage temperatures. No illness was reported among food handlers. Microbial analysis of food samples showed presence of faecal coliforms in low numbers, which suggested unhygienic handling of some food items, although no food-borne pathogens were isolated. Nursing home staff were given instructions for controlling the outbreak, including an emphasis on handwashing and

**FIGURE 4****EPIDEMIC CURVE, OUTBREAK 1, NOVEMBER 1998****FIGURE 5****EPIDEMIC CURVE, OUTBREAK 2, NOVEMBER 1998**

general hygiene, and isolating affected residents and the staff caring for them where feasible.

Stool samples collected toward the end of the outbreak were forwarded to SEALS for testing. All five samples were found to be positive for NLVs by RT-PCR and negative for other pathogens. Thus, it was considered that the outbreak was caused by person-to-person spread of NLVs.

#### **Outbreak 2**

On 24 November, a second nursing home (also in Sydney's south) reported an outbreak of gastroenteritis with onset of the first case on 20 November and the last case on 24 November. Of 104 residents, 38 had been unwell with diarrhoea, vomiting and abdominal cramps lasting five

to 30 hours. Three members of staff reported a similar illness. Due to the pattern of cases (a small number, initially increasing, then declining) a food source was considered unlikely, and it was decided not to conduct a food investigation (Figure 5). No connection with the first outbreak was established.

A general inspection of the nursing home was carried out and a lack of handwashing facilities identified, which may have had a bearing on the propagation of the outbreak. As for the previous outbreak, instructions were given concerning measures to control transmission of infection.

Stool samples were collected from six cases on 25 and 26 November and forwarded to SEALS for testing. Five were

found to be positive for NLVs by RT-PCR. It was again considered that the outbreak was caused by person-to-person spread of NLVs.

## DISCUSSION

Although many outbreaks of gastroenteritis may go unreported, public health authorities in NSW and elsewhere are required to respond to those outbreaks that are notified. These tend either to follow specific meals, catered functions or holiday camps, or to occur in residential or semi-closed institutions such as hospital wards, aged-care facilities and child-care centres. The purpose of the public health investigation is to determine a source, minimise further spread and guide affected individuals to appropriate health care.

Ideally, the public health response consists of an epidemiological investigation and a food-environmental investigation, both of which may be supported by microbiological testing. The epidemiology may suggest a point-source outbreak (based on finding a single illness peak and the fact that cases were exposed to one event or food item), person-to-person spread (in which new cases continue to occur over a period of time and there is no single risk factor for illness), or a combination of these mechanisms (where secondary cases may follow an initial peak). NLVs have been implicated both in point-source outbreaks (food- or water-borne) and those resulting from person-to-person spread.<sup>7,8</sup>

In many investigations of gastroenteritis outbreaks, the probable pathogen is inferred from the pattern of illness, and many outbreaks of NLV-SRSVs have been established in the past on this basis,<sup>9</sup> or with the retrospective assistance of electron microscopy, radioimmunoassay or serological studies.<sup>10</sup> More recently, the RT-PCR assay has been applied to such investigations. For example, a Dutch study conducted in 1996 found that 60 of 69 outbreaks were caused by SRSVs, the majority in nursing homes and hospital wards.<sup>7</sup> In a US survey of outbreaks of non-bacterial gastroenteritis conducted in 1996-97, NLVs were detected by RT-PCR in 86 of 90 outbreaks.<sup>8</sup>

The ready availability of a rapid turnaround RT-PCR assay for local NLV strains in public health microbiology laboratories should allow the identification of an aetiological agent in an increasing proportion of gastroenteritis outbreaks. A definitive diagnosis may be important in these investigations. Because of the ability for NLVs to persist in the environment,<sup>11</sup> NLV outbreaks may require additional and rigorous infection control measures to prevent recurrent outbreaks (for example, in cruise ships or child-care centres). In instances of water or food contamination, the knowledge that NLVs are the

pathogens may help in both identifying the mechanism of contamination and in eliminating the source.

Although a relatively small number of stool samples (5-6) were collected during the outbreak investigations described here, and invariably at the end of the outbreak, the RT-PCR assay detected NLV RNA sequences in nearly all samples. The assay is extremely useful in these settings. The RT-PCR-based assay for NLVs, which is now available statewide through the SEALS Virology Laboratories, is a significant addition to the armamentarium for prompt public health response to gastroenteritis outbreaks.

## REFERENCES

1. Murphy AM, Grohmann GS, Christopher PJ, Lopez WA, Davey RG, Millsom RH. An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. *Med J Aust* 1979; 2: 329-333.
2. Stafford R, Strain D, Heymer M, Smith C, Trent M, Beard J. An outbreak of Norwalk virus gastroenteritis following consumption of oysters. *Commun Dis Intell* 1997; 21: 317-320.
3. Wilson R, Anderson LJ, Holman RC, Gary GW, Greenberg HB. Waterborne gastroenteritis due to the Norwalk agent: Clinical and epidemiological investigation. *Am J Public Health* 1982; 72: 72-74.
4. Cauchi MR, Doultree JC, Marshall JA, Wright PJ. Molecular characterisation of Camberwell virus and sequence variation in ORF 3 of small round-structured (Norwalk-like) viruses. *J Med Microbiol* 1996; 49: 70-76.
5. Green SM, Dingle KE, Lambden PR, Caul E, Ashley CR, Clarke IN. Human enteric Caliciviridae: A new prevalent small round-structured virus group defined by RNA-dependent RNA polymerase and capsid diversity. *J Gen Virol* 1994; 75: 1883-1888.
6. Hansman G. Detection of gastroenteritis viruses by electron microscopy and RT-PCR. Honours thesis, University of NSW, 1998. Unpublished.
7. Vinje J, Altena SA, Koopmans MPG. The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in The Netherlands. *J Infect Dis* 1997; 176: 1374-1378.
8. Fankhauser RL, Noel JS, Monroe SS, Ando T, Glass RI. Molecular epidemiology of 'Norwalk-like viruses' in outbreaks of gastroenteritis in the United States. *J Infect Dis* 1998; 178: 1571-1578.
9. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. *Am J Public Health* 1982; 72: 1329-1332.
10. Blacklow NR, Cukor G. Norwalk virus: A major cause of epidemic gastroenteritis. *Am J Public Health* 1982; 72: 1321-1323.
11. Cheesbrough JS, Barkess-Jones L, Brown DW. Possible prolonged environmental survival of small round structured viruses. *J Hosp Infect* 1997; 35: 325-326. ☒