PREVALENCE AND GENOMIC VARIATION OF NORWALK-LIKE VIRUSES IN CENTRAL AUSTRALIA IN 1995–1997

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Summary. – Norwalk-like viruses (NLVs) have now been found to be important causes of gastroenteritis amongst infants and young children as well as older children and adults. Although detected, such viruses appeared not to be a major cause amongst infants and young children hospitalized with gastroenteritis in Alice Springs, central Australia over the period January 1995–December 1997. Nine NLV-positive cases were identified amongst stools from 360 different patients. From the nine cases however, eight different NLV strains were identified from comparisons of the sequence of a section of the RNA polymerase gene, and a high degree of genomic diversity was evident amongst them. In general, these strains were more similar to those identified in other countries than to those identified in central Australia over the three year period. Of the strains identified,six (and most probably seven) were classified in genogroup I, while only one was classified in genogroup II. This predominance of genogroup I strains is in contrast to most of the more recent findings made elsewhere, including those made in other parts of Australia. Phylogenetic analysis indicated that the central Australian strains spanned a range of known representative NLV strains, with one of the genogroup I strains showing a 96% nucleotide identity to Saratoga virus.

Key words: Australia; genomic variation; Norwalk-like viruses; prevalence

Introduction

NLVs have long been recognized worldwide as one of the major causes of epidemic non-bacterial gastroenteritis in adults and older children. Although, particularly more recently, they have been described to be associated with gastroenteritis in infants and young children as well (Lew *et al.,* 1994a; Matsuno *et al.,* 1997; Bon *et al.*, 1999; Parks *et al.,* 1999). NLVs, which are also referred to as small round structured viruses (SRSVs), are small , single-stranded positive RNA viruses and have recently been designated as a genus in the *Caliciviridae* family, based on phylogenetic analyses and genome organization (Table 1). They are distinguishable morphologically from the classical caliciviruses by electron microscopy (Caul 1996).

The enteric infections with NLVs are usually self-limiting and characteristically associated with acute diarrhoea, nausea and vomiting, and may be accompanied by fever, headache or myalgia. The viruses are highly infectious and secondary spread arising from person-to-person transmission is common (Kapikian *et al.*, 1996). NLV outbreaks have usually been described to occur in settings involving often large groups of people in nursing homes, hospitals, schools, hotels, camps and cruise ships. Large point source outbreaks of NLV infections have been associated with faecally contaminated food, water and shellfish (Kapikian *et al.*, 1996). In Australia, the settings in which NLV infections have been found to be involved are entirely similar to those generally described elsewhere in the world (Wright

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Abbreviations: CTAB = cetyltrimethylammonium bromide; EIA = enzyme immunoassay; NLV(s) = Norwalk-like virus(es); NV = Norwalk virus; nt = nucleotide; RT-PCR = reverse transcription–polymerase chain reaction; SDS = sodium dodecyl sulfate; SRSV(s) = small round structured virus(es)

Table 1. New structure of the *Caliciviridae* family approved by the International Committee on Taxonomy of Viruses*

Genus	Type species	Other species
Lagovirus	Rabbit hemorrhagic disese virus	European brown hare syndrome virus
"Norwalk-like viruses"	Norwalk virus	–
"Sapporo-like viruses"	Sapporo virus	–
Vesivirus	Vesicular exanthema virus of swine	Feline calicivirus

*Data from Green *et al.* (2000).
The names in quotation marks are provisional names.

et al., 1998). However, although widely studied as a virus group relatively little has so far been published on the molecular biology of NLVs circulating over time within defined regions (Maguire *et al.*, 1999).

We have analyzed for antibodies to Norwalk virus (NV) a total of 247 sera collected in 1977, 1984 and 1986 from patients of all ages admitted to Alice Springs Hospital, central Australia,the vast majority without gastroenteritis. For this we used recombinant NV antigen obtained through the courtesy of Dr M.K. Estes,Baylor College of Medicine, Houston, TX, USA in an enzyme immunoassay (EIA) and found that over 96% of sera from patients over the age of two years and approximately 70% of sera from patients up to the age of two years showed the presence of such antibodies (unpublished data).This suggested overall widespread exposure to NV, and also very probably to at least closely related NLVs (Noel *et al.*, 1997; Hale *et al.*, 1998), and confirmed and extended the results of the similar but smaller such survey carried out by Parker *et al.* (1994) using 52,1977 central Australian sera. Therefore, in the light of the antibody survey results and as reports on outbreaks of NLV-associated gastroenteritis in Australia have not included non-urban settings generally and central Australia specifically, we investigated the incidence of NLVs as a possible cause of gastroenteritis in infants and young children in central Australia over a three year period, through admissions to Alice Springs Hospital. This survey was also designed to determine how important NLVs were as a possible cause in those cases of infantile gastroenteritis in Alice Springs Hospital for which a causative agent has not been determined.

Materials and Methods

Stool specimens were obtained from infants and young children admitted to Alice Springs Hospital with gastroenteritis from January 1995 to December 1997 inclusive. Alice Springs Hospital serves an area of central Australia within an approximately 500 km radius of Alice Springs and a total population of about 25,000. Only stool specimens that were negative for rotavirus, astrovirus and adenovirus were used. These were tested for these viruses by commercial EIA (for rotavirus), reverse transcription–polymerase chain reaction (RT-PCR) (for astrovirus) and electron microscopy (for adenovirus and rotavirus). A total of 360 specimens – 134 from 1995, 110 from 1996 and 116 from 1997 – were tested. The age of the patients ranged from 2 weeks to 5.5 years with the majority under 2 years.

Viral RNA was extracted and purified from 400 µl of approximately 20% stool suspension following the method of Jiang *et al.* (1992). Briefly, this involved genetron extraction and polyethylene glycol precipitation of virus, followed by proteinase K, sodium dodecyl sulfate (SDS) and a cetyltrimethylammonium bromide (CTAB) treatment before phenol-chloroform and finally chloroform only extraction. The RNA was ethanol precipitated at -20°C and after pelleting and vacuum drying it was resuspended in 20 µl of sterile distilled water and stored at -80°C until used.

RT-PCR. For detection of virus by RT-PCR previously published primers that targetted sections of the NV/NLV RNA polymerase gene were used. The primers included NV3 and NV51 (Moe *et al.*, 1994) for both genogroup I and II viruses, and primers 4779 and mod51 (Wright *et al.*, 1998) for more specific targetting of genogroup II viruses. The RT-PCR conditions used were those of Jiang *et al.* (1992) as modified by Moe *et al.* (1994), although for PCR an annealing temperature of 50°C rather than 42°C was used occasionally with no change in results.

Agarose gel electrophoresis. Reaction products were visualized as bands in 2.5% agarose gels relative to size markers and known NV cDNA from RT-PCR amplification. This was kindly supplied by Dr G. Grohman, AWT, Sydney, Australia. The known NV initially, and later known NLVs served as positive RT-PCR controls, while sterile water in place of viral RNA was used as a negative control.

Southern blot analysis. Bands at approximately 206 nucleotides (nt) were confirmed as being of NLV origin by Southern blot analysis or occasionally by sequencing. For hybridization, the bands were transferred from agarose gels to nylon membranes by alkaline transfer and then probed using the 206 nt NV cDNA labeled with digoxigenin, following the manufacturer's instructions (Boehringer Mannheim,). Formamide was not used, the hybridization temperature was 55°C and the two final washes of the blots were done with 0.1x citrate saline plus 0.1% SDS at 55°C. Occasionally, less stringent conditions with a hybridization temperature of 50°C and/or final washing of the blots with 1x citrate saline plus 0.1% SDS at 50°C or 55°C were employed.

Nucleotide sequencing. RT-PCR products (cDNA) from samples giving a positive reaction with the NV cDNA probe were excised from the bands in agarose gels and further purified using the QIAEX II DNA Purification Kit (Qiagen) according to the manufacturer's instructions. Manual sequencing was carried out using the Thermo Sequanase Radiolabeled Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech) after a pretreatment of the purified cDNA samples with the enzymes from the Amersham Pharmacia Biotech PCR Product Presequencing Reagent Kit according to the manufacturer's instructions. Sequences were generated in both directions with the primers used for the RT-PCR and a consensus sequence for each of the strains

was deduced. A strain was defined as such when the sequence differed from the others by at least one nucleotide. Identification of sequences in GenBank with the greatest homology to seven of the strains identified in this study and sequence analysis for multiple sequence alignment and phylogenetic tree determination was carried out using the Hitachi DNASISv2.5 (Clustal 4) DNA and protein sequence analysis program. Nucleotide sequences for these seven strains (CANLV2-CANLV8) were compared with prototype and representative strains from the two NLV genogroups: NV (GenBank Acc. No. M87661), Desert Shield virus (U04469), Saratoga virus (U07614) and Southampton virus (L07418) from genogroup I, and Snow Mountain virus (L23831), Hawaii virus (U07611), Lordsdale virus (X86557), Melksham virus (X81879) and Toronto virus (U02030) from genogroup II.

Table 2. Details of the NLV isolation study in 1995–1997

Year (No. of stool specimens tested)	No. of NLV-positive specimens (%)	NLV strain	Date of sample collection	Age of patient
1995 (134)	3 (2.2%)	CANLV1	1/3/95	20 months
		CANLV2	21/8/95	22 months
		CANLV3	21/12/95	12 months
1996 (110)	5 (4.5%)	CANLV4	10/1/96	12 months
		CANLV5	24/4/96	16 months
		CANLV6	16/5/96	17 months
		CANLV6	27/6/96	3.25 years
		CANLV7	28/9/96	3.5 years
1997 (116)	1 (0.9%)	CANLV8	10/5/97	9 months

NLV = Nowalk-like virus.

Results

After RT-PCR with primers NV3/NV51 or 4779/mod51 and confirmation by Southern blot analysis and sequencing of the 206 nt PCR product, NLV was identified in 9 of the stool specimens from 360 infants and young children admitted to Alice Springs Hospital with gastroenteritis from January 1995 to December 1997 inclusive. The age of the children from whom the NLV-positive stools came ranged from 9 months to 3.5 years. In 1995, NLV was identified in 3 of the 134 stool specimens (2.2%), in 1996 in 5 of 110 specimens (4.5%), and in 1997 in 1 of 116 specimens (0.9%) (Table 2). NLV-positive cases appeared not to be limited to particular seasons.

From the sequence of the 166 nt segment of the 206 nt RT-PCR product that did not correspond to the primers used it was determined that the NLVs detected could be designated as 8 different strains (CANLV1-CANLV8, Table 2). The nucleotide sequences of 2 of the 1996 identifications (from 2 different individuals and collected 6 weeks apart) were identical and consequently were considered the same strain. Strains CANLV3 and CANLV4 were also very similar with only a 2 nt difference between them (Fig. 1).

The nucleotide sequence identity between the 7 strains CANLV2-CANLV8 and NV ranged from 67% to 99% (Table 3). As would be expected, the amino acid sequence identity was slightly higher owing to synonymous variations occurring, with identity ranging from 74% to 100% (Table 3). Data from strain CANLV1 was not included in Table 3 as too short sequence was obtained.

It was determined from comparison of the deduced amino acid sequences of the 166 nt segments from the NLV strains (data not shown) with the consensus-deduced sequences for genogroups determined by Wright *et al.* (1998), as well as

Table 3. Sequence identity (%) of a region of the RNA polymerase gene amongst the central Australian NLV strains and NV

Virus[a]	Year of isolation							
	1995			1996				1997
	NV	CANLV2	CANLV3	CANLV4	CANLV5	CANLV6	CANLV7	CANLV8
NV		89	91	91	71	95	87	85
CANLV2	80		91	91	79	89	96	93
CANLV3	77	77		100	76	95	89	87
CANLV4	78	77	99		79	95	89	87
CANLV5[b]	67	68	69	68		79	74	76
CANLV6	80	74	77	77	69		87	87
CANLV7	77	86	76	75	67	80		89
CANLV8	80	92	72	72	68	74	81	

[a]CANLV1 strain is not included because of a too short sequence obtained.
[b]CANLV5 was the only one of the central Australian strains classified into genogroup II.
Numbers at bottom left indicate results of a pairwise comparison of a 166 nt region (126 nt in the case of CANLV5) of the RNA polymerase gene. Numbers at top right indicate results of pairwise comparison of the deduced amino acid sequences of the above regions. Sequence identity is the percentage of the exact matches of nucleotides or amino acids.
NV = Norwalk virus, the prototype strain for genogroup I.

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                                                                                                                  80
      NV AGGTGATTATGTCATCAGGG TCAAAGAGGGGCTGCCATCT GCATTCCCATGTACTTCCCA GGTGAACAGCATAAATCACT
  CANLV7 cggtgattatgtgatcagcg tcaaagatggtctaccatct ggtttcccatgcacctcaca agtgaacagcatcaaccatt
  CANLV8 aggcgattatgtgatcactg taacatagggcctgccatct ggctttccatgcacttcaca ggtgaatagcatcaaccact
  CANLV2 aggtgattatgtgatcagtg ttaaagatggtctaccatct ggctttccatgcacttcaca ggtgaatagcatcaaccact
      SV TGGCGATTATGTGATCAGTG TTAAAGATGGTCTGCCATCT GGCTTTCCATGCACTTCACA GGTGAATAGCATCAACCACT
  CANLV6 tggagattatgtcatcaggg tcgcagagggtctcccatct ggtttcccatgcacatcaca agttaacagtataaaccatt
     SHV TGGCGACTACGTCATAAGAG TGAAGGAAGGCCTCCCATCT GGTTTCCCATGCACATCACA AGTTAATAGTATAAACCATT
  CANLV4 tggggactacatcataaggg ttaaagaaggtctcccctca ggctttccctgtacttcaca agttaacagtataaatcact
  CANLV3 tggggactacatcataaggg ttaaagaaggtctcccatca ggctttccctgtacttcaca agttaacagtataaatcact
Cons-GI -GG-GA-TA--T-AT-A--G T-----A-GG-CT-CC-TC- GG-TT-CC-TG-AC-TC-CA -GT-AA-AG-AT-AA-CA-T
                                                                                                                  40
      TV                                                GGTGTGCCTTGCACCTCACA GTGGAACTCCATTGCCCACT
  CANLV5                                                ggatacccatgtacttcaca atggaactccatcgctcact
     MKV                                                GGCGTCCCTTGCACCTCTCA ATGGAATTCCATTGCACACT
     SMV                                                GGAGTACCCTGCACATCACA GTGGAATTCCATCGCCCACT
      LV                                                GGTGTGCCCTGCACCTCTCA ATGGAATTCCATCGCCCACT
Cons-GII                                                GG----CC-TG-AC-TC-CA -TGGAA-TCCAT-GC-CACT
                                                                                                                 166
      GGATAATTACTCTCTGTGCA CTGTCTGAGGCCACTGGTTT ATCACCTGATGTGGTGCAAT CCATGTCATATTTCTCATTT TATGGT
      ggatctacactttgtgtgcc ctatcagaagtcactggttt ggctcctgatgtaatacagt cacagtcttacttttcattt tatggt
      ggatcctaactctatgtgca ctatcagaagtcactggctt gtcccctgatgtgatacaat cacaatcttatttctcattt catggt
      ggatcctgactctatgtgca ctgtcagaagtcactggctt gtcccctgatgtgatacaat cacaatcttacttctcattt tatggt
      GGATCCTAACTCTATGTGCA CTGTCAGAAGTCACTGGCTT GTCCCCTGATGTGATACAAT CACAATCTTATTTCTCGTTT TATGGT
      ggttaataactttgtgtgcc ctttctgaagtaactggtct gtcgccagatgttatccagt ccatgtcatatttctctttc tatggt
      GGTTAATAACTCTGTGCGCC CTTTCTGAAGTGACTGGCCT GTCGCCAGACGTTATCCAAT CCATGTCATATTTCTCTTTC TATGGT
      ggctaattaccctctgtgcc ctctctgaggttactggact ctctcctgatgtaatacagt cacagtcatacttctcattt tatggt
      ggctaatcaccctctgtgcc ctctctgaggttactggact ctctcctgatgtaatacagt cacagtcatacttctcattt tatggt
      GG-T----AC--T-T--GC- CT-TC-GA-G--ACTGG--T --C-CC-GA-GT--T-CA-T C----TC-TA-TT-TC-TT- -ATGGT
                                                                                                                 126
      GGTTGCTTACTCTGTGTGCC CTTTCTGAAGTTACAGGACT AGGCCCCGACATCATACAAG CTAATTCTATGTACTCTTTC TATGGT
      ggcttctcacccttttgtgca ctttctgaagtcacggacct gtctcctgacattatccagg ccaactccctgttttctttc tatggt
      GGCTCCTCACCCTATGTGCT CTTTCTGAAGTCACAGACCT TTCTCCTGACATTGTGCAGG CCAACTCCCTTTTCTCCTTT TATGGT
      GGCTCCTCACACTCTGTGCA CTATCTGAAGTCACAAACCT GGCTCCTGACATCATACAAG CTAACTCCTTGTTCTCTTTC TATGGT
      GGCTCCTCACTCTCTGTGCA CTCTCTGAAGTTACAAACCT GTCCCCTGACATCATACAGG CTAATTCCCTCTTTTCCTTC TATGGT
      GG-T-CT-AC-CT-TGTGC- CT-TCTGAAGT-AC----CT ----CC-GACAT--T-CA-G C-AA-TC--T-T--TC-TT- TATGGT
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Fig. 1

Multiple nucleotide sequence alignment of central Australian NLV strains CANLV2 – CANLV8 with reference strains

NV = Norwalk virus; SV = Saratoga virus; SHV = Southampton virus; TV = Toronto virus; MKV = Melksham virus; SMV = Snow Mountain virus; LV = Lordsdale virus. The sequences represent a 166 nt region of the viral RNA polymerase gene in the case of the upper group of 9 strains which are members of genogroup I, and a 126 nt region in the case of the lower group of 5 strains which are members of genogroup II. nt 1 corresponds to nt 4693 (4733 for the genogroup II strains) of NV. Cons-GI and Cons-GII indicate consensus sequences for genogroups I and II, respectively. Upper case letters indicate the sequences of the reference strains and consensus sequences and the lower case letters the sequences of the central Australian NLV strains.

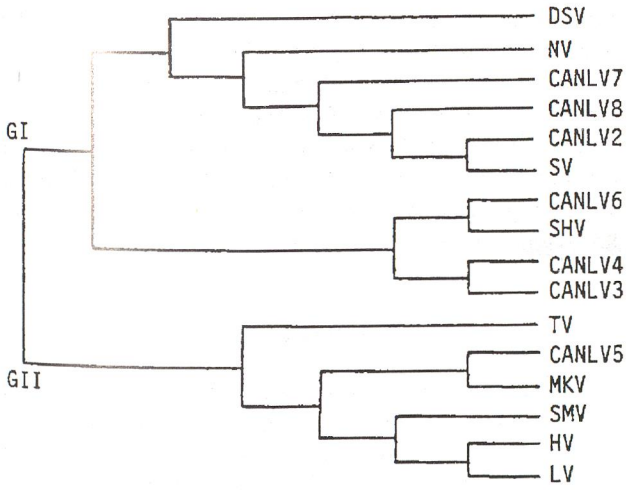

Fig. 2
Phylogenetic tree showing clustering relationships among central Australian NLV strains CANLV2-CANLV8 and reference strains from genogroups I and II

The tree is based on a 166 nt region (126 nt region in the case of CANLV5 and the other genogroup II strains) of the viral RNA polymerase gene, corresponding to nt 4693–4858 (4733–4858 for genogroup II strains) of NV. Genogroup I (GI): DSV (Desert Shield virus), NV (Norwalk virus), SV (Saratoga virus), SHV (Southampton virus). Genogroup II (GII): TV (Toronto virus), MKV (Melksham virus), SMV (Snow Mountain virus), HV (Hawaii virus), LV (Lordsdale virus). Generated using the DNASIS v2.5 sequence analysis program.

by phylogenetic analysis (Fig. 2) that all but one of the strains could be classified within genogroup I. CANLV5 strain was the only one to be classified in genogroup II (Table 4). Although only a 126 nt sequence could be obtained for this strain it fitted clearly into genogroup II on the basis of both of the above methods of determination. The determination of CANLV1 as a genogroup I strain is considered indicative only as too short sequence was obtained (data not shown). It should be noted that CANLV5 strain could be reversely transcribed, amplified and sequenced using NV3/NV51 primers, and that the cDNA was detected by the NV cDNA probe via Southern blot analysis, although only under lower conditions of stringency.

Sequences from none of the central Australian NLV strains were fully identical to any of those listed in GenBank. The nucleotide identity ranged from 86% to 96%; the 96% identity was between CANLV2 strain and Saratoga virus (Table 4). The multiple sequence alignment and phylogenetic analysis indicated that the 6 central Australian strains CANLV2-CANLV8, classified as genogroup I strains, spanned a range of the prototype and representative strains – NV, Desert Shield virus, Saratoga virus and Southampton virus (Figs.1 and 2). These viruses were chosen to represent a wide range of genetic diversity in this genogroup. Strains CANLV2, CANLV7 and CANLV8 appeared to be most closely related to Saratoga virus,while CANLV6 strain, and more distantly CANLV3 and CANLV4 strains were most closely related to Southampton virus. CANLV5 strain was most closely related to Melksham virus amongst the prototype and representative viruses chosen from genogroup II – Snow Mountain virus, Hawaii virus, Lordsdale virus, Melksham virus and Toronto virus (Figs. 1 and 2).

Discussion

NLVs appeared not to be a major cause of gastroenteritis in hospitalised infants and young children in central Australia in the period 1995–1997. The relatively low number of positive cases identified in this three year period was slightly surprising given the previously identified high level of circulating antibody to NV in the region (unpublished data). The EIA as used in that study would most probably also have detected antibodies against at least most of the genogroup I NLVs as well (Hale *et al.*, 1998). Although the antibody survey had been carried out 9 years prior to the NLV survey it would not have been expected for antibody prevalence to have changed substantially, given that such antibody levels remained at the same high level from 1977 to 1986 (unpublished data). A possible consideration could be that, as is usually the case with adults and older children, such episodes of gastroenteritis were generally not severe enough for the children to be admitted to hospital; a more

Table 4. Strain sequences obtained from GenBank with the closest match to those of the central Australian NLV strains

NLV strain (central Australia)	Genogroup	Strain from GenBank with closest match (% identity), GenBank Acc. No.	Origin of closest match strain, reference
CANLV2	I	Saratoga virus (96%) U07614	Military ship, Lew *et al.*, 1994b
CANLV3	I	UK2/12121/89 (93%) S71765	Adult, sporadic-UK, Ando *et al.*, 1994
CANLV4	I	UK2/12121/89 (93%) S71765	Adult, sporadic-UK, Ando *et al.*, 1994
CANLV5	II	Mur1-1997-JP (88%) AB019269	Direct submission, 1998
CANLV6	I	Human calicivirus RNA polymerase gene (92%) U56394	Sewage-New Zealand, Direct submission, 1996
CANLV7	I	Maryland virus (86%) U07612 Cts1-1995-JP (86%) AB019262	Adult, nursing home-USA, Lew *et al.*, 1994b Direct submission, 1998
CANLV8	I	Cts1-1995-JP (95%) AB019262	Direct submission, 1998

community based study would be required to establish whether this may be the case. We have no explanation for the obvious discrepancy, but it may reflect the use of different experimental approaches. The two sets of results are therefore not comparable.

A high degree genomic diversity was observed amongst the NLV strains identified in this survey. In fact in most cases they were more similar to strains identified in other countries than to the others identified in central Australia, suggesting the possible entry of new strains from outside the region. Alice Springs and central Australia generally are relatively major national and also international tourist destinations in Australia. The degree of genomic diversity and number of different strains is perhaps also not surprising considering the large geographic area which Alice Springs Hospital serves and therefore from which patients in the survey reported on here were drawn. The finding of a high degree of genomic diversity amongst the central Australian NLV strains is in keeping with general findings made elsewhere, particularly when sequences of strains identified from large or different geographic areas have been compared (Kapikian *et al.*, 1996; Noel *et al.*, 1997; Wright *et al.*, 1998). Although many studies on NLV-caused gastroenteritis have focussed on individual and sporadic outbreaks, another study that was carried out in a defined area, amongst infants in a Brazilian shanty town over a period of 16 months, also found a high degree of genomic diversity to be present amongst NLV strains identified as causes of gastroenteritis (Parks *et al.*, 1999).

In the study reported on here two different sets of primers were used to maximize the ability to detect NLVs of genogroups I and II. It was found that over the 3 years of the survey the prevalence of NLV strains from genogroup I (6) was substantially higher than those from genogroup II (1). This is in contrast to the findings in several recent surveys, in which predominantly genogroup II NLVs were found to be the cause over a number of different gastroenteritis outbreaks (Wright *et al.*, 1998; Bon *et al.*, 1999; Maguire *et al.*, 1999). In fact, in southeastern Australia, where Wright *et al.* (1998) reported on a large number of different gastroenteritis outbreaks attributed to NLVs/human caliciviruses which had occurred over the period 1980–1996, genogroup II strains were found to be by far the most common. Genogroup I NLVs (and classical caliciviruses) were identified rarely. Therefore there appears to be a difference between central and southeastern Australia with respect to the incidence of NLVs. Parks *et al.* (1999) in their study of NLVs in a Brazilian shanty town have found a more even distriution between genogroups I and II, with genogroup I strains the more prevalent (6) compared to genogroup II strains (3).

It is also of interest to note that the "global" NLV strain for 1995/96 was determined by Noel *et al.* (1999) to be from genogroup II, from the Lordsdale virus cluster. The only genogroup II strain identified in our central Australian survey fitted into the Melksham virus (Snow Mountain virus) cluster, more distant from the Lordsdale virus cluster. Overall therefore, although not of major importance as a cause of gastroenteritis in hospitalized infants and young children in central Australia, the NLV strains circulating in this region appear to be unique to the region.

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