Molecular Detection of Human Calicivirus in Young Children Hospitalized with Acute Gastroenteritis in Melbourne, Australia, during 1999

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Reverse transcription-PCR and sequence analysis identified caliciviruses in 32 of 60 stool specimens (negative for other enteric pathogens) obtained from children admitted to our hospital with acute gastroenteritis. The overall annual incidence rate for calicivirus was 9% (32 of 354 children). Molecular analysis identified 30 “Norwalk-like virus” genogroup II (predominantly Lordsdale cluster) and 2 “Sapporo-like virus” strains.

Human caliciviruses are the most common cause of nonbacterial gastroenteritis outbreaks in adults and older children worldwide (2, 13) and have been estimated to cause 95% of outbreaks of food-related viral gastroenteritis in the United States, representing millions of gastroenteritis episodes each year (8). Little is known about the importance of these viruses as causes of sporadic acute gastroenteritis in young children requiring admission to a hospital (5).

Human caliciviruses can be divided into two genera: “Norwalk-like viruses” (NLVs) and “Sapporo-like viruses” (SLVs) (2). NLVs are a genetically diverse group of viruses that can be classified phylogenetically into two distinct genogroups: genogroup I, which includes Norwalk virus, and genogroup II, which includes Lordsdale and Snow Mountain viruses. NLVs are the major cause of outbreaks and sporadic gastroenteritis in adults (2, 13). SLVs have been associated mainly with gastroenteritis in nonhospitalized children (9). The importance of calicivirus infection in child health in general is still largely unknown.

The objectives of this study were to determine the minimum prevalence of human caliciviruses in stools from young children admitted to the Royal Children’s Hospital, Melbourne, Australia, during 1999 and to characterize the types and distribution of calicivirus strains identified.

A total of 354 stool specimens were collected from all children under the age of 5 years. Routine diagnostic tests identified rotavirus (71.5%), astrovirus (5.7%), adenovirus (3.7%), and other pathogens (including bacterial and parasitic pathogens [2.8%]). The remaining specimens (n = 60), where no etiologic agent was identified, were tested for the presence of calicivirus using two reverse transcription-PCR protocols that amplify either a 319- or 321-base fragment (P289 and P290 primers) or a 213-base fragment (degenerate primer set) of the RNA polymerase gene (2, 6).

A total of 32 of 60 samples were positive by one or both of the reverse transcription-PCR methods. The nucleotide sequences of these products were determined by direct cycle sequencing and analyzed using E-CLUSTAL W (available through the Australian National Genomic Information Service, University of Sydney).

Strains segregated genetically into three distinct NLV genogroup II clusters (n = 30) and two clusters of the SLV genera (n = 2). The majority of the genogroup II strains (26 of 30) belonged to the Lordsdale cluster, while three of the remaining strains (RCH 99-8, -10, and -18) were assigned to the Snow Mountain cluster and one was assigned to the rare Hillingdon cluster (RCH 99-27). Two strains (RCH 99-6 and -19) belonging to the SLV genera exhibited most similarity to the Manchester and Sapporo clusters, respectively.

We conducted phylogenetic analysis of 22 strains for which a 220-nucleotide sequence was obtained using the P289-P290 primer pair (Fig. 1). These strains clearly segregated into three distinct NLV genogroup II clusters (Lordsdale, Snow Mountain, and Hillingdon) and two SLV clusters (Fig. 1). The strains not included in this analysis were those for which sequence information was obtained solely using the degenerate primer pair (strains RCH 99-7, -11, -16, -17, and -29) and those for which less than the 220-nucleotide sequence was obtained using the P289-P290 primer pair (strains RCH 99-2, -4, -10, -24, and -30).

This study highlights the importance of calicivirus as a causative agent of sporadic cases of acute gastroenteritis in young children who require hospitalization. The overall annual incidence rate of calicivirus infection during 1999 in Melbourne children under 5 years of age was 9% (32 of 354 children). This figure represents a minimum incidence rate, since only fecal specimens negative for other enteric pathogens were examined. The occurrence of mixed infections remains unknown. Our study shows good agreement with previous surveys of the incidence of calicivirus infection in children hospitalized with acute gastroenteritis in France, China, and South Africa (1, 11, 12). In comparison, surveillance studies of young children with mild diarrhea from community or outbreak settings detected calicivirus in 19 to 88% of such cases in various countries, including Finland, Canada, and Mexico (3, 4, 9, 10).
The extent of genetic diversity in strains isolated from children admitted to our hospital was consistent with results for adults from whom over 40 distinct strains have been identified from more than 70 outbreaks in the United States and Australia (2, 13). Our findings that NLV genogroup II strains were the dominant type (identified in 94% of positive specimens) is in agreement with those of previous studies of NLV infections in pediatric populations in France, Finland, and Canada (1, 4, 9). Most genogroup II strains identified in our study (84%) belonged to the Lordsdale cluster, which has a worldwide distribution and is prominent in many countries, including Australia, the United States, and Canada (2, 4, 13). The rare Hillingdon genogroup II cluster has been identified previously only in outbreak specimens from Japan (GenBank accession number AB020558), Canada (4), and Norway (GenBank accession number x89034). Overall, the divergence in NLV strains identified in the pediatric population in Melbourne appears to be a reflection of the genetic diversity present in the broader general community.

SLV strains appear uncommon as a cause of severe gastro-

FIG. 1. Phylogenetic tree of sequences of a 220-nucleotide region of the RNA polymerase gene (open reading frame 2) of 22 calicivirus isolates collected in Melbourne during 1999 and 16 reference strains available in GenBank. Distances were calculated by the Jukes-Cantor method, available in the MEGA analytical package (7), and trees were drawn using the neighbor-joining method. Reference strains were representative of clusters from both the NLV and SLV genera. The GenBank accession numbers for the reference strains are as follows: for Snow Mountain, NCRNAPBM1; for Camberwell, U46500; for Lordsdale, x86557; for Hawaii, HCU07611; for Mexico, HCU22498; for Melksham, x81579; for Desert Shield, DSU04469; for Hillingdon, AB020558; for Southampton, L07418; for Norwalk, M87661; for KY89, L23828; for Houston, U95644; for Manchester, x86560; for Sapporo, HCU65427; and for London, U95645.
enteritis in young children requiring hospitalization, being detected in only two samples in this study, perhaps reflecting the belief that SLV-associated gastroenteritis is less severe than NLV-associated gastroenteritis (9, 10). SLVs were also relatively uncommon (9 of 154 specimens) as a cause of outbreaks or of sporadic gastroenteritis in adults and older children not requiring hospitalization in Melbourne from 1986 to 1996 (13).

In conclusion, our results show that human caliciviruses, predominantly genogroup II of the NLVs, are an important cause of sporadic cases of acute gastroenteritis in infants and young children requiring hospitalization in Melbourne, Australia, during January to December 1999. Further epidemiological studies are required to identify whether the predominance of NLVs of genogroup II observed in this 1-year study persists over a longer period.

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