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Annual Incidence, Serotype Distribution, and Genetic Diversity of Human Astrovirus Isolates from Hospitalized Children in Melbourne, Australia

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The incidence of astrovirus infection in children under 5 years of age hospitalized for acute gastroenteritis in Melbourne, Australia, during 1995 was determined. Astrovirus was detected in 16 fecal specimens by Northern (RNA) dot blot analysis of RNA isolated from feces with an astrovirus-specific cDNA probe. The incidence of astrovirus infection was determined as 4.2% (16 of 378 total samples) compared with rates of 63.2, 3.7, and 4.2% for rotavirus, adenovirus, and all bacterial pathogens, respectively. Astrovirus was detected during the winter season and mainly in infants between 6 and 12 months of age. Serotyping of samples was carried out by reverse transcriptase PCR and direct sequencing of a 348-bp region of the capsid protein gene. Type 1 strains predominated (11 of 13 typeable samples), although type 4 isolates were also detected. Astrovirus was retrospectively identified in 13 fecal samples collected from hospitalized infants between 1980 and 1985 and shown to contain small viruses by electron microscopy. Type 1 isolates were again the most common, although a type 5 strain was also found. Comparative sequence analysis indicated that type 1 astroviruses exhibited up to 7% sequence divergence over a 15-year period; however, all mutations were silent. The incidence of astrovirus reported here indicates that the virus is a significant cause of severe diarrhea in young children. The genetic analysis also provides important molecular epidemiological information relevant to the development of preventative therapies.

Astroviruses were first detected in fecal samples from children with diarrhea by visualization of virus particles by electron microscopy (EM) (1, 15). The agent is now clearly associated with gastrointestinal illness both in volunteers ingesting virus filtrates and in natural infection in the community (7). Although astrovirus is commonly associated with mild disease of short duration, symptoms in children are often as severe as those observed with rotavirus diarrhea (7). Nosocomial astrovirus infections have also been reported (3, 4, 22).

Epidemiological surveys of agents isolated from young children with diarrhea attending outpatient clinics, admitted to the hospital or investigated in longitudinal community surveys, indicate that astroviruses are a significant cause of acute gastroenteritis, with prevalence rates of 2.7 to 8.6% (2, 7–9, 19). Astrovirus was also detected in 6 to 10% of fecal samples from children with diarrhea in which no bacterial or viral agents were identified (26), while an attack rate of 89% was recently determined in an astrovirus-associated diarrhea outbreak in a day care center (18). The incidence of astrovirus determined in surveys reflects the diagnostic assay employed, with the enzyme immunoassay (EIA) exhibiting greater sensitivity than EM. Genetic methods (hybridization and reverse transcriptase PCR [RT-PCR]) have been developed and evaluated but not yet applied to large-scale epidemiological surveys (11, 19, 28). However, the sensitivity of RT-PCR detection has been shown to be greater than that of the EIA (18). Surveys of antibody prevalence indicate that 70% of children are exposed to astrovirus during the first 5 years of life (13).

The astrovirus virion is composed of a single nonenveloped capsid layer 28 nm in diameter, while the viral genome is a single-stranded positive-sense polyadenylated RNA 6.8 to 7.6 kb in length (7, 10, 27). The genome contains three open reading frames (ORFs): ORF1a and ORF1b, which encode the viral protease and polymerase; and ORF2, which encodes the capsid precursor (10, 27). A subgenomic RNA that is colinear with the 3′ end of the genome and contains ORF2 has been identified in the cytoplasm of infected cells (17, 20, 21).

Human astroviruses are currently classified into seven serotypes by reactivity of capsid proteins with polyclonal sera and monoclonal antibodies (12, 14). Recently, sequence analysis of the capsid gene (ORF2) identified a 348-bp region that is conserved in viruses of the same serotype but varies between strains of different serotypes as determined by EIA (23). Thus, a correlation between genotype and serotype has been established.

Limited information is available about the extent of genetic variation in astrovirus clinical isolates, especially in relation to seasonal variation occurring in isolates collected from children hospitalized with gastroenteritis. Such information is important in the context of the future development of preventative therapies. In this report, we describe the incidence, monthly occurrence, and distribution of serotypes of astroviruses isolated from children under 5 years of age admitted to the Royal Children's Hospital in Melbourne during 1995. In addition, the extent of sequence variation in these isolates was determined and compared with those of isolates collected 10 to 15 years earlier and isolates causing outbreaks of gastroenteritis in the Melbourne community in 1995.

MATERIALS AND METHODS

Viruses and fecal samples. Standard human astrovirus (HAstV) types 1 to 7 grown in CaCo-2 cells were kindly provided by John Kurtz, John Radcliffe Hospital, Oxford, United Kingdom, and stored at −70°C. Fecal samples were collected from children under 5 years of age within 48 h of admission with acute gastroenteritis to the infectious diseases ward at the Royal Children's Hospital in Melbourne, Australia, in 1995. Astrovirus-positive samples isolated from children admitted to the Royal Children's Hospital between 1980 and 1985 or from children involved in gastroenteritis outbreaks in Melbourne in 1995 (provided by John Marshall, Victorian Infectious Diseases Laboratory, Fairfield Hospital, Melbourne, Australia) had been detected by EM examination of negatively

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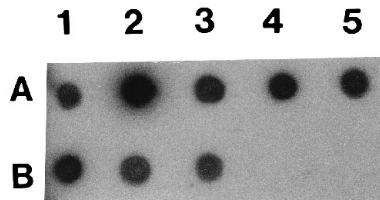

FIG. 1. Northern hybridization of RNA extracted from HAstV types 1 to 7 (A1 to A5 and B1 and B2), an astrovirus-positive fecal sample (B3), a rotavirus-positive fecal sample (B4), and a negative fecal sample (B5) with a cross-serotype DIG-labeled probe derived from HAstV 1 to 7.

stained fecal extracts. Fecal samples, as 10% (wt/vol) homogenates or ultracentrifuged pellets (resuspended in Tris buffer) for the outbreak isolates, were stored at −70°C.

Isolation of astrovirus RNA, RT-PCR, and sequencing. RNA was isolated by phenol-chloroform extraction of tissue culture supernatants, fecal homogenates, or ultracentrifuged pellets; purified by adsorption to hydroxyapatite; and eluted in potassium-phosphate buffer (6). RT-PCR was carried out by the method of Gentsch et al. (5) and with the astrovirus-specific primers Mon269 and Mon270 (23). After gel purification of PCR-derived cDNA, direct cycle sequencing was carried out with the fmol DNA Sequencing System (Promega, Madison, Wis.) and the same primers.

Preparation of a cross-serotype astrovirus-specific probe. To prepare an astrovirus-specific probe to detect all astrovirus serotypes, RNA derived from each of the type 1 to 7 HAstVs was used to generate a 449-bp cDNA product by RT-PCR as described above. Each of the cDNAs was used as a template for second-round PCR with the same primers and with incorporation of digoxigenin (DIG)-11-dUTP (Boehringer Mannheim, Mannheim, Germany). The seven individual DIG-labeled cDNAs were gel purified, quantified, and blended so that the final concentration of each in hybridization (described below) was 10 ng ml^{-1}.

Northern RNA hybridization. Northern hybridization was carried out under stringent conditions (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 50°C). Total RNA from virus or fecal samples (5 μl) was denatured by being boiled for 3 min and spotted onto a positively charged nylon membrane (Boehringer Mannheim). RNA was fixed to the membrane by UV cross-linking for 5 min before prehybridization at 50°C for 4 h in a buffer consisting of 50% formamide, 7% sodium dodecyl sulfate (SDS), 50 mM sodium phosphate (pH 7), 2% blocking reagent (Boehringer Mannheim), 5× SSC, and 0.1% *N*-laurylsarcosine. Hybridization was carried out at 50°C in the same buffer, which also contained 10 ng of each DIG-labeled probe ml^{-1}. After being washed in 2× SSC–0.1% SDS at room temperature (twice) and 0.1× SSC–0.1% SDS at 50°C (twice), the bound probe was detected with anti-DIG antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and the chemiluminescent substrate CDP-*Star* (Boehringer Mannheim).

Nucleotide sequence accession number. The nucleotide sequences determined in this study have been deposited in the GenBank sequence database and have been assigned the accession numbers U49212 to U49220.

RESULTS

Epidemiology of astrovirus infection. Between January and December 1995, fecal specimens were collected within 48 h of admission from 378 children under 5 years of age admitted to the Royal Children's Hospital with acute gastroenteritis. Samples were examined by routine techniques for the presence of bacterial and parasitic pathogens and by EIA for the presence of rotavirus and adenovirus. A total of 239 samples were found to be positive for rotavirus and excluded from further investigation. Of the remainder, 113 were screened for the presence of astrovirus by Northern hybridization of RNA extracted from fecal homogenates with a cross-serotype astrovirus-specific probe. The specificity of the probe was determined with RNA derived from HAstV 1 to 7, an astrovirus-positive fecal sample, rotavirus-positive stool, and negative control stool. The probe hybridized only to astrovirus RNA and was equally sensitive in detecting all standard serotypes (Fig. 1). This analysis indicated the presence of astrovirus RNA in 16 fecal samples, representing 4.2% of the total number of samples. The rate of astrovirus detection was second only to that for rotavirus

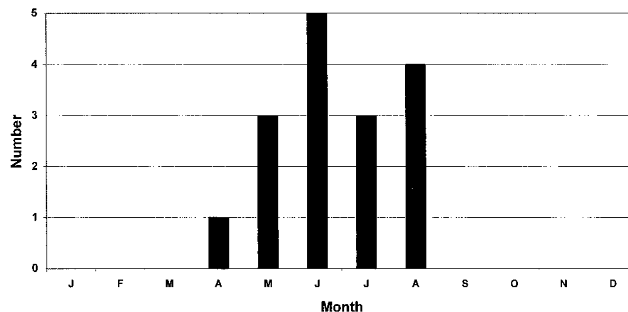

FIG. 2. Monthly occurrence of astrovirus in children under 5 years of age hospitalized with gastroenteritis at the Royal Children's Hospital, Melbourne, Australia, 1995.

(63.2%) and greater than those for adenovirus (3.7%) and bacterial pathogens (4.2% for all pathogens).

Subsequent review of patient records suggested that three patients were likely to have acquired astrovirus nosocomially, having been readmitted with diarrhea after recent discharge from the hospital where they had been treated for illness unrelated to gastroenteritis. In addition, one patient was subsequently found to have had simultaneous infection with astrovirus and *Clostridium difficile*. The remaining 15 astrovirus-positive samples had no other pathogen identified. Astrovirus was detected between May and August (Fig. 2) and was detected mainly in children between 6 and 12 months of age (7 of 16). Three of the patients were less than 6 months of age, three were between 1 and 2 years of age, and three were older than 2 years. During the major winter period (June to August), astrovirus was identified in 8% of samples.

Genotyping of 1995 astrovirus isolates. RNAs from the 16 astrovirus isolates identified above were subjected to RT-PCR with the astrovirus-specific primers Mon269 and Mon270 (23). Thirteen samples yielded the expected 449-bp cDNA, thereby confirming the hybridization results. Determination of the sequence of an internal 348-bp region was carried out with the 13 amplified specimens with the same primers. Comparisons with the sequences of standard astrovirus serotypes described in the literature indicated that the 13 Melbourne samples could be classified as either type 1 (11 isolates) or type 4 (2 isolates). On the basis of nucleotide sequence variation within these types, four subtypes of type 1 (designated RCH1A, RCH1B, RCH1C, and RCH1D) and two subtypes of type 4 (designated RCH4A and RCH4B4B) were identified (Table 1). Strain RCH1A was represented by six isolates, RCH1B and RCH1C were represented by two isolates each, and RCH1D was represented by a single isolate. The two type 4 isolates exhibited 1.1% sequence variation (4 bp differences), while the type 1 strains showed 0.3 to 0.9% sequence variation (1 to 3 bp differences [Table 2]). However, none of the nucleotide sequence changes translated into amino acid substitutions within each group. Strains RCH1A and RCH1B were associated with the three nosocomial infections.

The sequences of two astrovirus isolates collected from infants (6 and 12 months of age) during independent community outbreaks of gastroenteritis in Melbourne in 1995 were determined. Both isolates exhibited sequences identical to that of RCH1A.

Sequence comparisons with historical astrovirus isolates. Thirteen astrovirus isolates collected from children admitted to the hospital in Melbourne during 1980 and between 1982 and 1985 were investigated. Previous routine EM of fecal samples had indicated the presence of small viruses. The charac-

TABLE 1. Percentage identity in 348-bp region of ORF2 of astrovirus clinical strains and HAstV types 1 to 7[a]

Strain (Yr [s])	% Identity with HAstV type[b]						
	1	2	3	4	5	6	7
RCH1A (1995)	**90.5**	77.6	77.0	77.0	78.4	83.9	79.0
RCH1B (1995)	**90.5**	77.0	77.0	77.0	78.4	83.3	79.0
RCH1C (1995)	**91.1**	78.2	77.6	78.2	79.6	84.5	80.2
RCH1D (1995)	**91.2**	78.2	78.2	78.2	79.6	84.5	80.2
RCH1E (1980)	**96.8**	79.3	79.6	78.2	78.4	84.5	79.6
RCH1F (1982–1985)	**96.5**	79.3	79.9	78.7	78.7	84.8	79.9
RCH4A (1995)	77.9	79.6	79.0	**96.0**	77.3	80.5	79.9
RCH4B (1995)	77.9	79.9	78.2	**96.6**	77.3	81.3	80.5
RCH5 (1984)	77.3	73.3	78.2	78.2	**95.1**	76.7	81.3

[a] Sequences of standard strains were obtained from the GenBank database (accession numbers L06802, L23513, L38505, L38506, L38507, L38508, and U15136).
[b] Numbers in boldface indicate greatest identity with the specified strain.

teristic star-like appearance was not detected by EM, but all isolates were confirmed as astrovirus by RT-PCR. Nucleotide sequence analysis, as described above, indicated that this collection comprised three individual strains: two type 1 strains (designated RCH1E and RCH1F) and a type 5 strain (RCH5) (Table 1). Strain RCH1E was represented by the single 1980 isolate, while RCH1F was recovered from 11 patients between 1982 and 1985. RCH5 was isolated from a patient with severe combined immunodeficiency who developed diarrhea after admission to the hospital for a bone-marrow transplant and is therefore likely to have been acquired nosocomially.

Sequence comparisons between the type 1 astroviruses isolated in 1980 to 1985 and those circulating in 1995 indicated that significant temporal diversity existed. Strains RCH1E and RCH1F (isolated 2 years apart) exhibited 1.4% sequence variation; however, the degree of variation between RCH1E and the 1995 strains was between 5.8 and 7% (Table 2). Similarly, strain RCH1F exhibited 5.5 to 6.6% diversity compared with the 1995 strains (Table 2). However, although as many as 24 nucleotide differences were observed, none of the changes resulted in amino acid substitutions. Hence, astrovirus strains isolated from the Melbourne pediatric community appear to exhibit the steady accumulation of silent mutations. Nevertheless, diversity in the distribution of serotypes was apparent. Interestingly, only a single type 1 strain was identified in the 3-year period 1982 to 1985, while four type 1 strains appear to have cocirculated in 1995.

DISCUSSION

In this report, we have described the incidence, monthly occurrence, and serotype distribution of astroviruses isolated in Melbourne during 1995. Our data indicate that astrovirus was responsible for 4.2% (16 of 378) of the total number of gastroenteritis infections requiring admission of children under 5 years of age to the hospital investigated during 1995. Astroviruses were the sole enteric pathogen identified in 15 of the 16 positive samples (94%), and the virus was identified during the winter season, mainly in children between 6 and 12 months of age. The detection of astrovirus in children with diarrhea requiring hospitalization indicates that the virus can cause severe disease.

TABLE 2. Percentage identity in 348-bp region of ORF2 of type 1 astrovirus clinical strains

Strain (Yr [s])	% Identity with:					
	RCH1A	RCH1B	RCH1C	RCH1D	RCH1E	RCH1F
RCH1A (1995)	100					
RCH1B (1995)	99.4	100				
RCH1C (1995)	99.7	99.1	100			
RCH1D (1995)	99.7	99.7	99.4	100		
RCH1E (1980)	93.0	93.0	93.7	94.2	100	
RCH1F (1982–1985)	93.4	93.4	94.0	94.5	98.6	100

The incidence determined here concurs with that determined by a survey of infectious agents causing diarrhea in Australian children in 1978 to 1983 which showed an overall incidence of astrovirus of 2.17% and an incidence of 4.3% in children under 5 years of age (8). In contrast, a survey of specimens collected from the United States, Korea, and Peru indicated a rate of occurrence of 3.5% for astrovirus (19), while surveys from Guatemala (2) and Thailand (9) detected astrovirus in 7.3 and 8.6% of samples, respectively. Results from the earlier Australian survey, carried out by examination of fecal samples by EM (8), imply that detection by hybridization and EM can be equally sensitive techniques. However, comparison of the sensitivities of EM and hybridization in the same laboratory suggests that hybridization is more sensitive than EM for the detection of astrovirus in stools (28). Hybridization, however, appears to be more sensitive than RT-PCR, since only 13 of 16 samples positive by dot blot analysis were able to be amplified. In addition, the dot blot method proved to be highly specific, since none of the hybridization-negative samples tested yielded a positive RT-PCR result. The reason for RT-PCR's inability to amplify all positive samples may reside in the presence of inhibitors of RT-PCR in the fecal sample-derived RNA.

The distribution of serotypes of astrovirus isolates in Melbourne in 1995 (as determined from nucleotide sequence data) showed a predominance of type 1 strains (85%). This is the dominant serotype worldwide (12, 23). Nucleotide sequence differences in a 348-bp region of the capsid gene permitted subdivision of type 1 isolates into four subtypes which are likely to be distinct genetic strains. In addition to the cocirculation of genetically different type 1 strains, type 4 strains were simultaneously present. The genetic complexity of cocirculating strains differs from the findings of Noel et al. (23), who demonstrated that there was a clustering of strains with the same sequence circulating in various locations worldwide. Clustering of genetically identical strains was apparent, however, in strains collected in Melbourne during 1982 to 1985 which exhibited identical type 1 sequences. Whether this represents a change in the genetic epidemiology of the virus over time or is due to the restricted sample size examined in 1982 to 1985 is unknown.

Of particular interest was the identification of the first Australian strains belonging to the rare types 4 and 5. These serotypes exhibited a prevalence of 10 and 2%, respectively, in a survey which included samples from various locations worldwide (23). A survey of samples collected over 10 years in the Oxford region showed incidences of 6 and 5%, respectively (12), while a 17-year survey in the same region indicated a low incidence of these types (14). In contrast, type 4 had an incidence of 15% of typeable isolates in the 1995 survey described here, and the type 5 isolate was one of five randomly selected samples from 1984.

The rate of temporal genetic variation observed in astrovirus was similar to that seen in the VP7 capsid protein gene of rotavirus isolates collected in Melbourne over a particular winter epidemic season, i.e., <1% (24). However, astroviruses exhibited a greater rate of accumulation of mutations over

extended periods of time (10 years or more) than did rotavirus (maximum of 7% sequence variation for astrovirus, maximum of 3.4% for rotavirus) without any accompanying amino acid substitutions. Similar temporal accumulation of mainly silent mutations has been observed for the capsid protein genes of foot-and-mouth disease virus over a 6-decade period (16). The rate of accumulation of mutations in astrovirus also appears to be greater than that recently determined for Ross River virus (25), in which isolates collected over a 33-year period exhibited a maximum of 4.9% sequence divergence. The region of the astrovirus capsid gene investigated in this study is not known to contain any immunoreactive epitopes of the virus (17), so no conclusion about the degree of antigenic stability of the virus can be reached. Therefore, further sequence information about the astrovirus capsid gene is required before a detailed analysis of mutation rates and antigenic variation can be made.

The limited survey of astrovirus isolates responsible for community outbreaks of gastroenteritis carried out here indicated that the same strain caused sporadic cases resulting in hospitalization of children. Further analysis of outbreak isolates may yield information about the extent of strain variation in the total pediatric community of Melbourne.

In this report, we have determined that astrovirus has an incidence of 4.2% in children admitted to the hospital for acute gastroenteritis. This rate was second to that of rotavirus but greater than those of enteric adenoviruses and common bacterial pathogens during 1995. However, this rate is probably an underestimate of the incidence of astrovirus infection, since not all rotavirus-negative fecal samples were tested because of limited fecal material. In addition, simultaneous infection with rotavirus and astrovirus is possible, and rotavirus-positive specimens were excluded from analysis. Since a pathogen remains unidentified in a large proportion of samples collected (>20%), further investigation of possible etiologic agents, e.g., calicivirus, is warranted.

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REFERENCES

1. **Appleton, H., and P. G. Higgins.** 1975. Viruses and gastroenteritis in infants. Lancet **i:**1297.
2. **Cruz, J. R., A. V. Bartlett, J. E. Herrmann, P. Caceres, N. R. Blacklow, and F. Cano.** 1992. Astrovirus-associated diarrhea among Guatemalan ambulatory rural children. J. Clin. Microbiol. **30:**1140–1144.
3. **Esahli, H., K. Breback, R. Bennet, A. Ehrnst, M. Eriksson, and K.-O. Hedlund.** 1991. Astroviruses as a cause of nosocomial outbreaks of infant diarrhea. Pediatr. Infect. Dis. J. **10:**511–515.
4. **Ford-Jones, E. L., C. M. Mindorff, R. Gold, and M. Petric.** 1990. The incidence of viral-associated diarrhea after admission to a pediatric hospital. Am. J. Epidemiol. **131:**711–718.
5. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B. K. Das, and M. K. Bhan.** 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1365–1373.
6. **Gouvea, V., J. R. Allen, R. I. Glass, Z.-Y. Fang, M. Bremont, J. Cohen, M. A. McCrae, L. J. Saif, P. Sinarachatanant, and E. O. Caul.** 1991. Detection of group B and C rotaviruses by polymerase chain reaction. J. Clin. Microbiol. **29:**519–523.
7. **Greenberg, H. B., and S. M. Matsui.** 1992. Astroviruses and caliciviruses: emerging pathogens. Infect. Agents Dis. **1:**71–91.
8. **Grohmann, G. S.** 1985. Viral diarrhoea in Australia, p. 25–28. *In* S. Tzipori (ed.), Infectious diarrhoea in the young. Excerpta-Medica/Elsevier, Amsterdam.
9. **Herrmann, J. E., D. N. Taylor, P. Echeverria, and N. R. Blacklow.** 1991. Astroviruses as a cause of gastroenteritis in children. N. Engl. J. Med. **324:**1757–1760.
10. **Jiang, B., S. S. Monroe, E. V. Koonin, S. E. Stine, and R. I. Glass.** 1993. RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. Proc. Natl. Acad. Sci. USA **90:**10539–10543.
11. **Jonassen, T. O., C. Monceyron, T. W. Lee, J. B. Kurtz, and B. Grinde.** 1995. Detection of all serotypes of human astrovirus by the polymerase chain reaction. J. Virol. Methods **52:**327–334.
12. **Kurtz, J. B., and T. W. Lee.** 1984. Human astrovirus serotypes. Lancet **ii:**1405.
13. **Kurtz, J. B., and T. W. Lee.** 1987. Astroviruses: human and animal, p. 92–107. *In* G. Bock and J. Whelan (ed.), Novel diarrhoea viruses, 1st ed. John Wiley & Sons, Chichester, United Kingdom.
14. **Lee, T. W., and J. B. Kurtz.** 1994. Prevalence of human astrovirus serotypes in the Oxford region 1976–1992, with evidence for two new serotypes. Epidemiol. Infect. **112:**187–193.
15. **Madeley, C. R., and B. P. Cosgrove.** 1975. 28 nm particles in faeces in infantile gastroenteritis. Lancet **ii:**451–452.
16. **Martinez, M. A., J. Dopazo, J. Hernández, M. G. Mateu, F. Sobrino, E. Domingo, and N. J. Knowles.** 1992. Evolution of the capsid protein genes of foot-and-mouth disease virus: antigenic variation without accumulation of amino acid substitutions over six decades. J. Virol. **66:**3557–3565.
17. **Matsui, S. M., J. P. Kim, H. B. Greenberg, L. M. Young, L. S. Smith, T. L. Lewis, J. E. Herrmann, N. R. Blacklow, K. Dupuis, and G. R. Reyes.** 1993. Cloning and characterization of human astrovirus immunoreactive epitopes. J. Virol. **67:**1712–1715.
18. **Mitchell, D. K., S. S. Monroe, X. Jiang, D. O. Matson, R. I. Glass, and L. K. Pickering.** 1995. Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase-polymerase chain reaction. J. Infect. Dis. **172:**1437–1444.
19. **Moe, C. L., J. R. Allen, S. S. Monroe, H. E. Gary, Jr., C. D. Humphrey, J. E. Herrmann, N. R. Blacklow, C. Carcamo, M. Koch, K.-H. Kim, and R. I. Glass.** 1991. Detection of astrovirus in pediatric stool samples by immunoassay and RNA probe. J. Clin. Microbiol. **29:**2390–2395.
20. **Monroe, S. S., B. Jiang, S. E. Stine, M. Koopmans, and R. I. Glass.** 1993. Subgenomic RNA sequence of a human astrovirus supports the classification of *Astroviridae* as a new family of viruses. J. Virol. **67:**3611–3614.
21. **Monroe, S. S., S. E. Stine, L. Gorelkin, J. E. Herrmann, N. R. Blacklow, and R. I. Glass.** 1991. Temporal synthesis of proteins and RNAs during human astrovirus infection of cultured cells. J. Virol. **65:**641–648.
22. **Nazer, H., S. Rice, and J. A. Walker-Smith.** 1982. Clinical associations of stool astrovirus in childhood. J. Pediatr. Gastroenterol. Nutr. **1:**555–558.
23. **Noel, J. S., T. W. Lee, J. B. Kurtz, R. I. Glass, and S. S. Monroe.** 1995. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. J. Clin. Microbiol. **33:**797–801.
24. **Palombo, E. A., R. F. Bishop, and R. G. H. Cotton.** 1993. Intra- and inter-season genetic variability in the VP7 gene of serotype 1 (monotype 1a) rotavirus clinical isolates. Arch. Virol. **130:**57–69.
25. **Sammels, L. M., R. J. Coelen, M. D. Lindsay, and J. S. Mackenzie.** 1995. Geographic distribution and evolution of Ross River Virus in Australia and the Pacific Islands. Virology **212:**20–29.
26. **Utagawa, E. T., S. Nishizawa, S. Sekine, Y. Hayashi, Y. Ishihara, I. Oishi, A. Iwasaki, I. Yamashita, K. Miyamura, S. Yamazaki, S. Inouye, and R. I. Glass.** 1994. Astrovirus as a cause of gastroenteritis in Japan. J. Clin. Microbiol. **32:**1841–1845.
27. **Willcocks, M. M., T. D. K. Brown, C. R. Madeley, and M. J. Carter.** 1994. The complete sequence of a human astrovirus. J. Gen. Virol. **75:**1785–1788.
28. **Willcocks, M. M., M. J. Carter, J. G. Silcock, and C. R. Madeley.** 1991. A dot-blot hybridization procedure for the detection of astrovirus in stool samples. Epidemiol. Infect. **107:**405–410.