A Prospective Case-Control Study of the Role of Astrovirus in Acute Diarrhea among Hospitalized Young Children

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This study examines the importance of astroviruses as a cause of acute diarrhea in hospitalized children <10 years old during a 5-year period. Stools were screened by electron microscopy and were tested for astrovirus, rotavirus, and enteric adenovirus by EIA. During the study, 14.6% of hospitalized children had diarrhea. Astroviruses were second only to rotaviruses as etiologic agents of both community-acquired and nosocomial diarrhea. Community-acquired astrovirus infection occurred in 6.8% of patients, and nosocomial disease occurred in 16.2%. Most cases occurred from March through June, and astrovirus type 1 was the most common. The symptoms of astrovirus-infected children were similar to those of children with rotavirus infection. However, astrovirus-infected children had a lower median age, less dehydration, and lower symptom severity scores and were less likely to have been admitted for gastroenteritis than were children with rotavirus. Astrovirus, for which only rehydration therapy is required, should be considered as another common diarrheal pathogen in children <2 years old.

In the United States, acute gastroenteritis is a common disease that is second only to acute viral respiratory tract infection in frequency among infectious diseases. In recent years, rotaviruses, enteric adenoviruses, calcicviruses, and astroviruses clearly have been associated with gastroenteritis in infants and young children. Many episodes of acute gastrointestinal illness still have no recognized cause but may be due to agents, such as astroviruses or calcicviruses, which are not yet routinely available.

Astroviruses are 28-nm, single-stranded RNA viruses that are part of the family Astroviridae [1]. Astroviruses cause diarrhea in several animal species and were first identified by use of electron microscopy (EM) in infants with gastroenteritis in 1975 [2]. The application of techniques such as EIA and reverse-transcriptase polymerase chain reaction (RT-PCR) [3–5] has shown astroviruses to be common enteric pathogens. Astroviruses have been identified as important agents of diarrheal disease among infants in day care [5, 6] and among hospitalized children [7, 8] and are important agents in nosocomial diarrhea in children’s hospitals [7, 9, 10] and in outpatient gastroenteritis [11]. The use of a sensitive EIA facilitates the prospective assessment of the importance of astrovirus infection in pediatric diarrhea by allowing the screening of a large number of samples. The objectives of this study were to determine the prevalence of astrovirus-associated diarrhea among a group of US children who were hospitalized with acute diarrhea or who acquired it during hospitalization, the antigenic types of astrovirus isolated from these children, and the clinical importance of astrovirus infection, compared with that of rotavirus and enteric adenovirus infections.

Materials and Methods

Study population. During a 5-year period (July 1988 through June 1993), children from birth to 10 years old who were admitted to 2 pediatric wards at Rhode Island Hospital were prospectively surveyed for diarrhea at admission and during hospitalization. From May 1991 through July 1992, we also enrolled control patients without diarrhea who were similar in age to and were admitted within 48 h of each case patient.

Diarrhea surveillance. A research nurse or study physician visited the pediatric wards each weekday and reviewed each child’s bedside chart, noting all children admitted with a diagnosis of diarrhea, vomiting, gastroenteritis, or acute dehydration. Children admitted on weekends and holidays were enrolled if still hospitalized on the next weekday. A stool specimen was requested from each child whose illness met the definition of diarrhea (see below). Clinical data were collected from each patient’s chart, including age, admission diagnosis, initial symptoms, duration of illness before hospitalization, and gastrointestinal symptoms while in the hospital.

Definitions. A “diarrheal episode” was defined as the occurrence within a 24-h period of ≥3 episodes of stools of a less-formed...
character than normal for that child, with no identifiable noninfectious cause for this symptom. A “new episode of diarrhea” was defined as the occurrence of diarrhea separated from a previous episode by ≥ 1 week. An “episode of nosocomial diarrhea” was defined as diarrhea in a hospitalized child with onset ≥ 72 h after admission, a time beyond the estimated incubation period of 1–3 days. Children with persistent diarrhea, defined as diarrhea lasting > 10 days, were excluded from the study. Dehydration was defined by the clinical scoring system described by Santosham et al. [12]. Severity of diarrhea was assessed by using a numerical scoring system modified from that of Flores et al. [13].

Astrovirus infection was defined as the detection of astrovirus antigen or the presence of astrovirus, as determined by EM, in a stool specimen from a child with diarrhea. A mixed infection was considered to have occurred when > 1 enteropathogen was identified in a single diarrhea episode.

Specimen collection and storage. Stool specimens were collected within 48 h of admission or at the onset of diarrhea in children who developed diarrhea while in the hospital. Specimens were stored at 4°C before testing and then were frozen at −70°C. Specimens that could not be processed or assayed within 48 h of being obtained were frozen at −70°C and then were thawed for subsequent testing.

Microbiologic tests. Monoclonal antibody-based EIAs were used to detect rotaviruses (Rotacclone; Meridian Diagnostics) and enteric adenoviruses (Adenclone 40/41; Meridian Diagnostics). All stool samples also were cultured on Graham 293 cells, as described elsewhere [14], to detect enteric adenoviruses. Group C rotaviruses were detected as described by Jiang et al. [15].

EM was done as follows: a 10% stool suspension was made in 2% ammonium acetate and 3 mM magnesium chloride (pH 7) and then was spun briefly at 15,000 g in a centrifuge (Eppendorf) to remove debris. A drop of supernatant was placed on a 400-mesh Formvar/carbon-coated copper grid. The grid was stained with 2% aqueous phosphotungstic acid (pH 6.5–7.0) for 2 min and then was blotted with filter paper. Grids were examined for virus particles by using a Phillips 300 electron microscope at a magnification of 52,000× for ~5 min.

Routine bacterial cultures for Salmonella, Shigella, Campylobacter, and Yersinia species and Escherichia coli O157:H7; parasitologic examination for ova and parasites; and EIA for Clostridium difficile were done, using standard microbiologic procedures, by the Clinical Microbiology Laboratory, at the discretion of the patient’s physician. From July 1992 through July 1993, stool specimens were examined for Cryptosporidium species by a modified Kinyoun acid-fast stain (Difco Laboratories).

Astrovirus detection. Astroviruses were detected by an indirect, double antibody, biotin avidin EIA, using reagents prepared by the Centers for Disease Control and Prevention. The EIA detects 8 types of astrovirus grown in tissue culture and has sensitivity and specificity values of 90% and 93%, respectively [16]. Specimens were considered to be positive if mean absorbance of the positive capture (P) and negative capture (N) wells met the following criteria: P/N ≥ 2 and P − N ≥ 0.07. Specimens were considered to have indeterminate results if either P/N ≥ 2 or P − N ≥ 0.07.

Specimens with positive or indeterminate EIA results and those with astrovirus visualized on EM were culture-adapted in Caco-2 cells by the method of Willcocks et al. [17], with the modification of Noel et al. [18], and were retested for astrovirus antigen by EIA. Astrovirus-positive samples were typed by EIA with antisera to astrovirus types 1–5 [18].

Statistical analysis. Demographic data and test results for astrovirus and other enteropathogens were entered into a data base (Panorama, version 3.0;1; Provue Development) on a Macintosh microcomputer (Apple Computer). Statistical results were analyzed using Statview (version 4.0; Abacus Concepts). The χ² test was used to test categorical variables, and analysis of variance tests were used to compare numerical variables. Nonparametric tests were used to analyze data without a normal distribution. All tests were 2-tailed and were considered to be significant when P < .05.

Results

From 1 July 1988 through 30 June 1993, 8743 children < 10 years old were admitted to 2 pediatric wards at Rhode Island Hospital. During the 5 years of the study, 1661 children (19%) had diarrhea at admission or developed nosocomial diarrhea. Of the 1661 children, 385 were excluded as not meeting the case definition of diarrhea because they had an underlying disease or antibiotic use thought to be the cause of their diarrhea, which left 1276 (14%) with diarrhea of presumed infectious etiology. Stool samples were not obtained from 469 children (27%) with diarrhea. At least 1 stool sample was collected from the remaining 807 children with diarrhea. Of these 807 children, 603 (75%) had diarrhea at admission, and 204 (25%) developed diarrhea during hospitalization. During the study period, 262 children were identified as control patients, and specimens were obtained from 141 (54%) of these children. Control patients were similar in age to patients with diarrhea (median age, 3 months vs. 4.8 months) and had a range of diagnoses, with the 3 most frequent being rule-out sepsis (40%), respiratory infection (32%), and admission for a surgical procedure (14%). Surveillance monitoring was conducted on 997 (81%) of 1239 planned surveillance days during the study period.

Table 1 shows the frequency of enteric pathogens in community-acquired diarrhea in the hospitalized children. Viruses were the most frequently isolated pathogens, with group A rotaviruses making up the largest percentage (30.0%), followed by astroviruses (6.8%), enteric adenoviruses (5.6%), and group C rotaviruses (3.3%). No caliciviruses were found. Only a subset of specimens was tested for enteric bacteria. This testing was done at the discretion of the physician only when findings such as bloody stool or high fever suggested that a bacterial etiology was likely. Of the stools tested, 21% were positive for enteric bacteria. Cryptosporidium were routinely sought only in the final year of the study and were found in 6.7% of stools tested. Mixed infections with 2 pathogens occurred in 26 episodes (4.3%) of community-acquired diarrhea.

Results of astrovirus testing. Astrovirus infection was detected in 60 patients by either EIA or EM. Both EIA and EM were done on stool specimens from 54 of the 60 patients: 21 (39%) specimens were positive by both assays, 24 (44%) were
positive by EIA only, and 9 (17%) were positive by EM alone. In these 9 specimens, the number of virus particles detected was small. Six of the 9 specimens were cultured, and 3 grew astrovirus. Sixteen specimens from the 24 patients positive by EIA alone were cultured, and astrovirus grew on 11. An additional 14 infected patients were identified by positive astrovirus culture results when the specimens from 26 patients with negative EM results and indeterminate results on EIA were tested.

**Astrtvirus-associated diarrhea.** Astrovirus was identified in 41 (6.8%) of the 603 episodes of community-acquired diarrhea and was the second most commonly identified individual agent of diarrhea after rotavirus (table 1). Coinfection with another pathogen occurred in 8 (1.3%) episodes and included 7 with group A rotavirus and 1 with salmonella. Yearly rates of astrovirus isolation ranged from 5.1% to 8.9% during the 5-year study (table 2). Astroviruses were identified more often than were enteric adenoviruses in the stools and accounted for almost one-fourth as many illnesses as rotaviruses. Astroviruses were significantly more likely to be detected in patients with diarrhea than in control patients (P = .0003).

Furthermore, astroviruses were second only to rotaviruses as a cause of nosocomial diarrhea. Astroviruses alone were identified in 24 (11.8%) of the 204 episodes of hospital-acquired diarrhea. Astrovirus coinfection occurred in another 9 (4.4%) episodes, all with group A rotavirus. These nosocomial astrovirus cases occurred 3–116 days (median, 10 days) after admission. Rotaviruses accounted for 27% of hospital-acquired cases, whereas enteric adenoviruses and mixed rotavirus-enteric adenovirus infections accounted for 1.5% and 2.9%, respectively. No bacterial agents were detected among the patients with hospital-acquired diarrhea.

**Seasonality.** To determine temporal trends, we assessed the dates of onset of diarrhea in patients with diarrhea on a monthly basis (figure 1). Rotavirus showed the expected peak for this region between January and May, with most cases of diarrhea occurring during the months of February and March. Enteric adenoviruses, on the other hand, had no seasonal predilection and occurred at low rates throughout the year. Over the 5 years, most astrovirus cases clustered during a period between March and June.

**Antigenic types.** A serotype could be identified for 17 (39%) of the 41 children with community-acquired astrovirus diarrhea; of the remaining children, the serotyping assay failed to identify a type for 1, there was an insufficient volume of stool for culture for 13, and stool specimens from 10 could not be adapted to culture in Caco-2 cells. Serotype 1 was most common (n = 12 [67%]) and was followed by type 2 (n = 2 [11%]), type 4 (n = 2 [11%]), and “not determined” (n = 1 [6%]). One patient (6%) had a mixed infection with serotypes 1 and 3. No type 5 infections were seen. Two of the serotype 1 infections were seen in children infected with astrovirus plus another pathogen.

Four antigenic types also were detected in the 33 children with hospital-acquired diarrhea. Nine (27%) of the stool specimens positive for astroviruses could not be serotyped because of insufficient volume of stool for culture (n = 4) or failure to be adapted to culture in Caco-2 cells (n = 5). Again, serotype 1 was most common (n = 16 [67%]), followed by type 2 (n = 1 [4%]), type 3 (n = 1 [4%]), type 4 (n = 1 [4%]), and “not determined” (n = 5 [21%]). Two serotype 1 infections and the serotype 2 infection were seen in children infected with astrovirus plus another pathogen.

**Age distribution.** Astrovirus infections occurred in all age groups but were most common in infants <3 months old (table 3). The median age of children with community-acquired astrovirus infection (3.4 months; range, 2 months to 6 years) was younger than the median age of 7.3 months (range, 1 week to 6 years) in rotavirus-infected patients. The median age of children with nosocomial astrovirus infection was 5.0 months (range, 2 weeks to 2 years) and did not differ significantly from the median age of 4.0 months (range, 1 week to 10 years) in rotavirus-infected patients.

**Severity of astrovirus-associated diarrhea.** To further assess the medical importance of astrovirus infections, the clinical findings of children infected with astrovirus alone were compared with findings for patients infected only with rotaviruses.
or enteric adenoviruses (table 4). Children with astrovirus infections were substantially less likely to be dehydrated or to have an admission diagnosis of gastroenteritis than those with rotavirus infection. In addition, the children with astrovirus infections had lower scores of disease severity than did rotavirus-infected patients.

Discussion

Diarrheal illness remains an important cause of hospitalization among US children. During a 5-year study, 14% of children hospitalized in a Rhode Island hospital had diarrhea as a cause of admission (11%) or as a cause of nosocomial illness (3%). Viruses were the most commonly identified cause, and astroviruses were second only to rotaviruses as a cause of community-acquired and of nosocomial infections. This study demonstrates that ≥40% cases of diarrhea among US children have a viral etiology, especially among children <2 years old in the late winter and spring. This finding has implications for diagnosis and treatment: since viral diarrheas are best treated with oral rehydration therapy, antibiotics can be withheld, and routine bacterial stool cultures have little use, except for those patients with symptoms that suggest a bacterial etiology (e.g., bloody diarrhea). Although gastroenteritis associated with astrovirus cannot be distinguished from other common diarrheal illnesses on clinical grounds, patients with astrovirus infection had lower rates of dehydration and lower severity scores than did those with rotavirus and were less likely to have been hospitalized with an admission diagnosis of gastroenteritis. Our finding agrees with those in other studies in which patients with astrovirus infections were found to be less likely to have dehydration [4] and more likely to be significantly younger than those with rotavirus infection [7, 19].

To our knowledge, this 5-year study is the first to search prospectively for astroviruses as a cause of diarrhea among US children hospitalized for diarrhea. Astroviruses were found to be second only to rotaviruses as etiologic agents of community-acquired diarrhea in our population of children, and they were significantly associated with diarrhea, being more common among children with acute community-acquired diarrhea (6.8%) than among control patients (0%). We also found that astroviruses were important agents in hospital-acquired cases of diarrhea in young children. Astroviruses were second only to rotaviruses as etiologic agents of nosocomial diarrhea, accounting for ~12% of cases and nearly half as many cases as rotavirus. This further confirms findings by others of the importance of astrovirus in hospital-acquired gastroenteritis [7, 9, 10, 20]. A study conducted in a day care center reported an

<table>
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<tr>
<th>Age</th>
<th>With astrovirus (n = 33)</th>
<th>With group A rotavirus (n = 159)</th>
<th>With enteric adenovirus (n = 26)</th>
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<tr>
<td>&lt;3 months</td>
<td>15 (45)</td>
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<td>20 (13)</td>
<td>4 (15)</td>
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<td>5 (15)</td>
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<tr>
<td>2–5 years</td>
<td>3 (9)</td>
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NOTE. Data are no. (%) of children. Children with coinfections were excluded.

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>With astrovirus (n = 33)</th>
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<th>With enteric adenovirus (n = 26)</th>
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<td>Stools per day</td>
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<td>3</td>
<td>5 (15)</td>
<td>11 (7)</td>
<td>2 (8)</td>
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<tr>
<td>4–5</td>
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<td>5 (19)</td>
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<td>19 (58)</td>
<td>103 (65)</td>
<td>19 (73)</td>
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<td>Days of diarrhea</td>
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<td>8 (5)</td>
<td>4 (15)</td>
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<td>2–4</td>
<td>16 (49)</td>
<td>81 (51)</td>
<td>11 (42)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>12 (36)</td>
<td>70 (44)</td>
<td>11 (42)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>23 (70)</td>
<td>125 (79)</td>
<td>19 (73)</td>
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<tr>
<td>Fever</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13 (39)</td>
<td>56 (35)</td>
<td>18 (69)</td>
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<td>&gt;38°C</td>
<td>17 (52)</td>
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<td>9 (35)</td>
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<td>87 (51)</td>
<td>12 (46)</td>
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<td>Admission diagnosis of gastroenteritis</td>
<td>16 (48)</td>
<td>114 (72)</td>
<td>17 (65)</td>
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</table>

NOTE. Data are no. (%) of children. Children with coinfections were excluded. *Enteric adenovirus was significant, compared with rotavirus and astrovirus (P < 0.05). **Astrovirus was significant, compared with rotavirus (P < 0.05).

attack rate of 89%, which suggests that astrovirus can be transmitted efficiently in a closed environment [21].

Our detection rates for astrovirus are similar to those reported among hospitalized children in other developed countries [7, 8, 11, 22, 23]. A previous survey for viral agents of gastroenteritis among US children found no astroviruses in 8 years of study [24]. Our results demonstrate that this study seriously underestimated the role of astroviruses. This underestimation was likely due to the use of EM, a detection method that is less sensitive than EIA, which was used in the current study.

A recent study has reported that caliciviruses are frequent in children with diarrhea [25]. The failure to detect caliciviruses in our population of children is similar to results from the survey done by Brandt et al. [24], who also used EM. EM is less sensitive than the RT-PCR assays now being used for calicivirus detection [26, 27]. The astrovirus detection rates reported in our study are also likely to be underestimates of the true prevalence of disease because the EIA that was used has been shown to be less sensitive than a PCR method that is now available [21].

As seen in previous long-term surveys [18, 19, 28, 29], most of our clinical isolates (67%) were serotype 1 astroviruses. Serotype 1 strains also were predominant in studies from children’s hospitals in England [30], Australia [8, 23], and the United States [7]. However, because typing by EIA detects only 5 of the 8 known serotypes and because a large number of isolates was not available for typing, it is difficult to draw firm conclusions about the frequency of serotypes in our community.

Prevention of astrovirus infections could decrease all hospitalizations by ~1% and hospitalizations for diarrhea by 7%. Astroviruses have been detected in outbreaks of disease associated with contaminated food and water; however, our results and those of other researchers suggest that astrovirus infections are ubiquitous, affecting most children in their first years of life. Preventive measures, such as improvements in hygiene and water, are therefore unlikely to have major impact on astrovirus disease. Immunization may have a role in the prevention of astrovirus gastroenteritis, since the decrease in disease prevalence with age suggests that protective immunity may develop against astrovirus diarrhea. Further epidemiologic studies are needed to assess the burden of astrovirus disease in outpatient and hospital settings and the serotypes in circulation, to determine whether vaccine development against astrovirus is feasible. Our results and those of other researchers suggest that the highest incidence of astrovirus infection occurs among children ≤ 6 months old; thus, if an astrovirus vaccine is developed, it will have to confer immunity very early in life.

We also note that, although our study establishes astrovirus as an important cause of gastroenteritis requiring hospitalization, an etiologic agent remained unidentified in 44% of hospitalized children with community-acquired diarrhea. Calicivirus has been recognized as a common cause of mild diarrhea and as a less common cause of severe diarrhea in children [25]. Further investigation of caliciviruses will be important to complete our understanding of the remaining known etiologic agents.

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References


