Prevalence of Astroviruses in a Children's Hospital

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SUNITA SHAISTRY, ANNE MARTIN DOANE, JAIME GONZALEZ,
USHA UPADHYAYULA, AND DORSEY M. BASS*
Department of Pediatrics and Center for Digestive Disease,
Stanford University, Stanford, California 94305-5208

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An enzyme immunoassay for astrovirus was used to screen 357 stool samples from 267 symptomatic inpatients at a tertiary-care children’s hospital. Thirty stool samples from 26 patients contained astrovirus antigen, while rotavirus was found in 34 samples and Clostridium difficile toxin was found in 40. Half of the astrovirus infections were nosocomial. Additional pathogens were identified in six of the astrovirus-positive stool samples. Most (80%) of the astroviruses recovered were of serotype 1. Astrovirus infections were significantly more common than rotavirus or C. difficile infections in very young infants and in those with surgical short-bowel syndrome.

Astroviruses are small, 28 to 34 nm, single-stranded RNA viruses which are part of a recently described family, Astroviridae (25). Astroviruses cause diarrhea in a variety of species and have been observed by electron microscopy in human infantile diarrheal stools since 1975 (1, 22). Recently, simple and sensitive assays such as enzyme-linked immunosorbent assays (EIAs) and reverse transcriptase-PCR have replaced electron microscopy and have shown astroviruses to be a common cause of diarrhea (7, 14, 24). Astroviruses have been increasingly identified as important agents of diarrheal disease in infants, immunocompromised patients (6, 12), and the elderly in nursing homes (11) and of nosocomial diarrhea in children’s hospitals (9, 10). Here we describe a new, sensitive, monoclonal antibody (MAb)-based EIA which detects all seven serotypes of human astrovirus and its application in surveillance of astrovirus among symptomatic patients at a tertiary children’s hospital in the United States.

MATERIALS AND METHODS

Stool samples. Stool samples were submitted for microbiologic analysis based on symptoms of vomiting, diarrhea, or feeding intolerance. A portion of each inpatient stool sample submitted to the Microbiology Laboratory at Lucile Salter Packard Children’s Hospital, Stanford, Calif., between 1 July 1996 and 1 July 1997 was aliquoted for this study. Samples were held at −70°C until the time of assay. For the EIAs, stools were suspended at 10 or 0.2% (wt/vol) in phosphate-buffered saline containing 15 to 20% fetal bovine serum (FBS) and pelleted at 1,000 × g for 5 min.

Antibodies. Polyclonal rabbit antiserum to cesium chloride-purified serotype 1 human astrovirus was prepared (3). Reference rabbit antiserum against human astrovirus serotypes 1 to 7 were kindly provided by J. B. Kurtz and T. W. Lee (Oxford, England). MAb 8G4 is an immunoglobulin G1 (IgG1) mouse hybridoma which reacts with human astrovirus serotypes 1 to 7 by immunoperoxidase staining of infected cells and by EIA (3). It was purified by protein A-Sepharose chromatography and biotinylated (30). MAb 191 is an IgG1 mouse hybridoma which recognizes all adenovirus serotypes, including 40 and 41. The initial astrovirus antigen EIA used rabbit polyclonal anti-serotype 1 human astrovirus serum as the capture antibody. A biotinylated MAb (8G4) directed against human astrovirus group antigen was used for detection. In preliminary experiments, this EIA was able to detect cell culture supernatants from all seven human astrovirus serotypes.

The second EIA used MAb 8G4 as the capture antibody and biotinylated 8G4 as the probe and TMB substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was used as the substrate and the plates were read on a Bio-Rad EIA reader at 450 nm after the reaction had been stopped with 1 M phosphoric acid. Cell culture-grown astrovirus and RRV served as positive and negative controls. All results were considered positive when the mean A450 was greater than twice that of the negative controls.

ROSAMI, Minitab 8.0 for Macintosh (Addison-Wesley, Reading, Mass.) was used to perform chi-square and Mann-Whitney nonparametric tests.

RESULTS

Assay development. Our initial astrovirus antigen EIA, similar to one previously described by Noel et al. (27), uses polyclonal rabbit serum as a capture antibody and provided a good signal-to-noise ratio both on tissue culture-grown virus and in mock-positive samples made by adding cell culture virus to stool samples. The sensitivity of the assay using purified serotype 1 astrovirus was approximately 10 to 20 ng/ml, and all seven human serotypes were detected equally well. RRV and reovirus serotype 1 consistently tested negative.

After assaying 334 clinical samples and finding 63 (18.8%) positive, we noted several problems with the assay. We found that many positive samples gave equally high absorbance read-
TABLE 1. Antigenic characteristics of astrovirus-positive stools

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Result of EIA based on:</th>
<th>Cell culture result</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit antiserum MAb 8G4 MAb 5B7</td>
<td></td>
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<tr>
<td>3</td>
<td>+ + NT + NT</td>
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<tr>
<td>386</td>
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* NT, not tested. Cell culture, adapted to cell culture on Caco 2 cells. Serotype, determined by microneutralization assay. + +, >200 OD units; +, >100 OD units; ±, equivocall; –, negative.

The 18 tissue culture-adapted viruses were tested by neutralization assay against serotype-specific polyclonal rabbit sera (Table 1). Fifteen (83%) of 18 were of astrovirus (nine samples), protozoans (five samples), or adeno-virus (four samples).

Thus, of the 267 symptomatic patients, 9.7% were positive for astrovirus, 11.6% were positive for RV, and 12.7% were positive for C. difficile. Other pathogens were identified in six (23%) of the astrovirus antigen-positive patients, including two RV and four C. difficile isolates. Thirteen astrovirus infections were considered to be nosocomial, as evidenced by a previous negative EIA during admission (8 patients) and/or onset of symptoms more than 72 h after admission (11 patients). The remaining 13 astrovirus infections were considered to be community-acquired.

Assessment of the duration of astrovirus shedding by four patients with astrovirus infections was possible. Two patients had shedding for less than 14 days, one had shedding for at least 10 days, and shedding by one was documented on three occasions over a span of 45 days.

**Patient age at infection.** When the ages of patients shedding astrovirus, RV, and C. difficile toxin were compared, it was evident that astrovirus infected a significantly younger population (median age, 0.7 year) than RV (median age, 3.0 years) or C. difficile (median age, 2.5 years) (Table 2). Patients shedding astrovirus were also significantly younger than the entire cohort of patients.

**Diagnosis at admission.** We examined the diagnoses at admission and the underlying diagnoses of patients identified with the three major pathogens. The most common diagnoses are presented in Table 3. The most striking finding is the association of astrovirus infection with short-bowel syndrome. Six of the 26 astrovirus-positive patients (including one who shed for at least 45 days) carried this diagnosis. It is unclear whether their underlying medical condition, their physical proximity on a surgical ward, or their panel of care providers explains this clustering. Very few (two) of the hematology/oncology patients shed astrovirus, perhaps as a function of their greater age and prior exposure. All three microorganisms appeared to be capable of causing diarrhea severe enough to warrant hospitalization in normal infants and children.

**Serotyping of isolates.** The 18 tissue culture-adapted viruses were tested by neutralization assay against serotype-specific polyclonal rabbit sera (Table 1). Fifteen (83%) of 18 were of astrovirus (nine samples), protozoans (five samples), or adeno-virus (four samples).

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<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Kidney/liver Tx+</th>
<th>BM Tx/cancer</th>
<th>Short bowel syndrome</th>
<th>Diarrhea</th>
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<tbody>
<tr>
<td>Astrovirus</td>
<td>3</td>
<td>2</td>
<td>6'</td>
<td>3</td>
</tr>
<tr>
<td>RV</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>C. difficile</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

* Tx, transplant.
* BM, bone marrow.
* P < 0.05 by chi-square test.
serotype 1, 2 were of serotype 2, and 1 was of serotype 3 by neutralization assay. The decreased susceptibility of older patients to astro-

^C. difficile^ iso-

variable reactivity of our serotype 1 isolates with MAb 5B7 and (80%) of our clinical isolates were serotype 1 astroviruses. Previous reports (19, 21).

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Astrovirus, RV, and ^C. difficile^ accounted for over 90% of the pathogens identified in our study, which included both community-acquired and nosocomial infections. We found that astroviruses accounted for approximately 10% of the diarrhea cases (21% of the identifiable cases) in this setting, which is comparable to previous reports (4, 10). We also observed an increased incidence of RV and astrovirus infections during winter months (data not shown), which is also consistent with previous reports (19, 21).

As seen in prior long-term surveys (20, 27), the majority (80%) of our clinical isolates were serotype 1 astroviruses. Serotype 1 strains were also predominant in two studies from children’s hospitals in England and Australia (26, 28). The variable reactivity of our serotype 1 isolates with MAB 5B7 and the isolation of three different serotypes demonstrate that multiple astrovirus strains were circulating in this children’s hospital during a single season.

Astrovirus infections occurred in a significantly younger pa-

^C. difficile^ infections in our studies. The decreased susceptibility of older patients to astro-

virus infection suggests that astrovirus-infected infants may develop protective immunity to resist subsequent exposures. Very little is known about such immunity, although it is known that young children develop serum antibodies to astroviruses early in life (15, 16, 17).

A novel observation in our study was the high rate of astrovirus infection in patients with short-bowel syndrome. Although this may have been due to ongoing environmental contamination or an unidentified carrier, other children on the same ward did not have as high an attack rate. These patients had undergone significant surgical intestinal resections and had lost not only absorptive surface area but large amounts of gut-associated lymphoid tissue, both of which losses may have rendered them particularly susceptible to symptomatic mucosal infection. Indeed, a 2 year old in our study with a very short gut excreted high titers of astrovirus for at least 45 days. Others have noted that patients with “underlying gastrointestinal disorders” were at increased risk for astrovirus-associated nosocomial diarrhea (9).

All three of the major pathogens identified in this study may be found in stools of asymptomatic pediatric patients. Examination of 20 control stool samples from a ward with the highest number of symptomatic astrovirus-shedding patients showed that 6 were positive, suggesting that asymptomatic astrovirus infection can be quite common (data not shown). Prior studies of day care center outbreaks have also shown that asymptomatic astrovirus shedding is common among older attendees (24). Further prospective case-control studies are warranted to fully assess the role of each of these pathogens in diarrheal disease of hospitalized pediatric patients.

ACKNOWLEDGMENTS

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We thank Suzanne Matsui for reagents and helpful discussions and David Kiang for assistance with ascites production. We also gratefully acknowledge the assistance of the nursing staff and the Department of Clinical Microbiology at the Lucile Packard Children’s Hospital.

REFERENCES


DISCUSSION

There is no widely accepted “gold standard” for detection of astroviruses in stool samples. Electron microscopy and cell culture isolation are expensive and cumbersome and may miss positive samples due to lack of sensitivity. On the other hand, highly sensitive assays based on EIA and PCR may be prone to false-positive results. In this report, we describe the develop-

ment of an EIA based entirely on a group-specific MAb which reacts with human astrovirus serotypes 1 to 7. The advantages of a MAb-based assay include unlimited supplies of consistent reagents and avoidance of possible detection bias due to the use of polyclonal serum raised to a single serotype of astrovir-

us. Our EIA appears to be reasonably specific, with 78% of positives confirmed by tissue culture isolation.

In our survey, a possible etiologic agent was identified in 45% of stool samples from symptomatic pediatric inpatients, similar to the 47% reported in a prior study of nosocomial diarrhea in a Swedish pediatric hospital (4). Previous studies of inpatient pediatric diarrhea from around the world have consis-
tently shown that viral infections, particularly those due to RV, account for the majority of cases (4, 8, 10, 13, 18, 31). Among bacterial pathogens, ^C. difficile^ is the most common cause of inpatient/nosocomial pediatric diarrhea (5, 23, 29).

Astrovirus, RV, and ^C. difficile^ accounted for over 90% of the pathogens identified in our study, which included both community-acquired and nosocomial infections. We found that astroviruses accounted for approximately 10% of the diarrhea cases (21% of the identifiable cases) in this setting, which is comparable to previous reports (4, 10). We also observed an increased incidence of RV and astrovirus infections during winter months (data not shown), which is also consistent with previous reports (19, 21).

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