Population-Based Prevalence of Symptomatic and Asymptomatic Astrovirus Infection in Rural Mayan Infants

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Symptomatic and asymptomatic astrovirus infection was prospectively determined in a 3-year birth cohort of Mayan infants. Stool samples from 271 infants and 268 older siblings were tested for astrovirus, adenovirus 40/41, rotavirus and Salmonella, Shigella and Campylobacter species. Concurrent diarrhea, vomiting, fever, or anorexia were noted. Astrovirus was detected in 164 infants (61%) and 20 siblings (7%). Rotavirus (4%) and adenovirus 40/41 (13%) were isolated less frequently. Of all diarrheal episodes reported at a visit, 26% (78/305) were associated with astrovirus; 17% (78/452) of astrovirus infections were associated with diarrhea and 9% with other symptoms. Only diarrhea was associated with astrovirus infection (odds ratio, 1.4; 95% confidence interval [CI], 1.07–1.92; \( P = .01\)). Of infants with astrovirus, 70% shed at multiple visits over a period of 2–17 weeks (median, 5). The point prevalence of astrovirus infection was significantly higher among infants than siblings (relative risk, 6.18; 95% CI, 3.93–9.72; \( P < .0001, \chi^2\)). Astrovirus was identified throughout the year, peaked in March and May, and decreased in September. In this population, astrovirus was the most common enteric pathogen isolated; symptomatic infection was prevalent among infants.

Astroviruses are second only to rotaviruses as a common cause of viral gastroenteritis in infants and young children worldwide [1]. Acute astrovirus gastroenteritis induces a mild, watery diarrhea that lasts for 2–3 days and may be associated with vomiting, fever, anorexia, abdominal pain, and constitutional symptoms that last up to 4 days. Protracted diarrhea and viral shedding are uncommon. Most astrovirus infections are detected in the winter months in temperate regions and in the rainy season in tropical climates, a pattern similar to that seen with rotavirus infections. Symptomatic infections are primarily found in infants and young children, particularly in developing countries, or in elderly, institutionalized populations.

Most data regarding astrovirus infection have been obtained from cross-sectional rather than longitudinal studies of children with gastrointestinal symptoms. Longitudinal data regarding the incidence of asymptomatic astrovirus infection have been described in US day care center studies [2, 3]; no longitudinal data have been published regarding children living outside of developed countries. In addition, astroviruses may be isolated from stool specimens from children with gastroenteritis that contain other enteric pathogens, making determination of the causative pathogen difficult.

As part of an oral poliovirus vaccine (OPV) immunogenicity study [4], we collected sequential stool samples from a 3-year population-based birth cohort of rural Mayan infants and their older siblings living in Chiapas, Mexico. We studied the prevalence of astrovirus, rotavirus, adenovirus 40/41, and Salmonella, Shigella, and Campylobacter species in this population and obtained information regarding the presence of common gastrointestinal symptoms around the time of sample collection. We therefore had the opportunity to explore the age-related prevalence and seasonality of infections with astrovirus and other enteric pathogens as well as the prevalence of asymptomatic astrovirus infection in this cohort of rural Mayan infants.

Methods

Study population. The study was conducted in the Mayan village of Navenchauc, 10 km from the city of San Cristobal de las Casas in Chiapas, Mexico’s southernmost state. Navenchauc consists of 629 households, with a population of 4000. The community is semiclosed, that is, although there is travel out of the village, most women and children do not leave the village. This setting allows for the assessment of circulation of viruses within the population.

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Consent for participation was obtained from parents of study infants. The study was approved by the Instituto Nacional de Diagnostico y Referencia Epidemiologicos in Mexico City and by the Committee for the Protection of Human Subjects at Stanford University.
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**Patient enrollment.** During a 3-year period (1992–1995), all healthy infants 6 weeks to 6 months of age living in the village, whose mothers consented, were enrolled in an OPV immunogenicity study. The study included a comparison of OPV given by mass versus routine administration. The mass OPV campaigns were conducted beginning each February and included two doses of OPV given 8 weeks apart; routine OPV administration was given at 2 and 4 months of age from July through December. Therefore, during the 3-year study period, enrollment of infants during the mass OPV campaign was offered to infants who were 6 weeks to 6 months of age at the start of the campaign, whereas infants enrolled during the routine OPV vaccination period were enrolled at 2 months of age.

All children received one dose of OPV at study weeks 1 and 9; stool samples were collected by home visits at study weeks 0, 1, 2, 4, 6, 8, 9, 10, 12, 14, and 17. To minimize recall bias, a history of fever, vomiting, anorexia, or diarrhea only in the 2 days prior to or on the day of sample collection was obtained at each home visit. Stool samples from up to 2 older siblings (2–5 years old) of each study infant were collected at study weeks 1, 9, and 17. No symptom history was obtained from siblings. Stool samples were collected in paper containers at the study subject’s home, taken to the field laboratory, and separated into 2 aliquots. One aliquot was tested immediately for Salmonella, Shigella, and Campylobacter species by standard methods [5–7]; the second was placed in 1.8-ml vials (Nunc, Roskilde, Denmark) and frozen at −20°C before shipment on dry ice to the virology research laboratory at Stanford University. Sibling stool samples were not tested for bacterial pathogens.

**Definition of symptoms.** Maternal reporting of the infants’ symptoms in the 2 days before or on the day of specimen collection was used to define all symptoms. Since a 3-day history of diarrhea could be obtained at the time of stool collection, but specimens were collected at 1- or 2-week intervals, the duration of an episode of each symptom was based on the following definition. Because visits were separated by 1- to 2-week intervals, symptoms identified at each visit were defined as separate episodes. Astrovirus infection associated with symptoms was considered definitive if the symptoms occurred concurrently or within 1 week before or after detection of an astrovirus-positive stool specimen.

**Laboratory methods.** All stool samples were tested for adenovirus 40/41, astrovirus, and rotavirus by immunoassay screening tests [8–12]. Rotavirus and adenovirus 40/41 were detected by a separate commercial ELISA kit for each pathogen (Rotaclone and Adenoclone 40/41, respectively, Cambridge Biotech, Worcester, MA; IDEIA Rotavirus and IDEIA Adenovirus, respectively, Dako Diagnostics, Cambridge, MA; IDEIA Rotavirus and IDEIA Adenovirus, respectively, Dako Diagnostics, Cambridge, MA). Positive and negative controls included in each kit were utilized, and positive samples were defined as $A_{405}$ of ≥0.150 for the Cambridge Biotech kits and $A_{450}$ of ≥0.1 + the average of the negative controls for the Dako Diagnostics kits.

Astrovirus antigen was detected by a noncommercial ELISA using antibody reagents provided by J. E. Herrmann (University of Massachusetts Medical Center) [9, 10]. Briefly, polyvinyl chloride ELISA plates were coated with astrovirus-specific monoclonal antibody 8E7 (1:5000 dilution in PBS, 100 μL/well) and incubated overnight at 4°C. Three washes with PBS, the plates were blocked with PBS/0.05% azide/2% fetal calf serum (FCS) (200 μL/well) and incubated overnight at 4°C or for 2 h at 37°C. The plates were again washed three times with PBS, and 40 μL/well PBS/azide/FCS and 10 μL/well stool suspensions (10% wt/vol) were added in duplicate. The plates were incubated overnight at 4°C and then for 1 h at 37°C. The plates were washed five times with PBS, and hyperimmune rabbit-anti-astrovirus antibody was added (1:1000 dilution in a solution containing 50% FCS/50% 0.1 M Tris [pH 7]/0.15% Tween 20; 50 μL/well). The plates were incubated for 1.5–2 h at 37°C and washed five times with PBS. Detector antibody, peroxidase-labeled goat anti-rabbit IgG (Bio-Rad, Richmond, CA; 1:3000 dilution in PBS/2% FCS; 50 μL/well) was added, and the plates were incubated for 1.5–2 h at 37°C. After five washes with PBS, 50 μL/well peroxidase substrate (ABTS) was added, and the plates were incubated in the dark for 10–15 min. The reaction was then stopped with 1% SDS (25 μL/well), and the plates were read at $A_{405}$ or $A_{414}$ in a plate spectrophotometer. Samples that exhibited absorbance values of ≥0.1 and ≥3 times the negative control were considered positive. According to these criteria, most positive samples had absorbance values of ≥0.2.

To confirm our ELISA results, we randomly selected 24 ELISA-positive and 24 ELISA-negative samples and tested for the presence of astrovirus RNA by reverse transcriptase–polymerase chain reaction (RT-PCR). RNA was extracted from fecal specimens using a published method [13] that uses guanidine thiocyanate and adsorption of viral RNA onto silica to minimize sample contamination with inhibitors of RT-PCR. To detect all seven known serotypes of human astrovirus, we used previously described primers and RT-PCR methods [14]. The sensitivity and specificity of the ELISA were 84% and 74%, respectively. Neither false-positive nor false-negative reactions were related to ELISA absorbance values. No attempt in this study was made to determine astrovirus serotype specificity among samples in which astrovirus antigen was detected.

**Statistical analysis.** The prevalence of symptomatic and asymptomatic astrovirus episodes was determined using the definitions described above. To control for incomplete stool sample collection from individual infants, point prevalence of astrovirus episodes was calculated for each study week. To control for an unequal number of stool specimens obtained from study infants and older siblings, the age-related prevalence of astrovirus infection was determined by comparing the point prevalence of astrovirus infection between study infants and older siblings for weeks when samples were collected from both infants and siblings each February and included two doses of OPV given 8 weeks apart; routine OPV administration was given at 2 and 4 months of age (weeks 1, 9, and 17). $x^2$ and Fisher’s exact (two-tailed) tests were used in analysis of discrete variables; analysis of variance (ANOVA) was used to compare means. Logistic regression analysis was used to determine the magnitude of effect, odds ratios, and significance of multiple factors on two outcome variables: overall prevalence of astrovirus infection and prevalence of symptomatic astrovirus infection. Variables with significant effects on either outcome variable at $P < .05$ in univariate analysis were included in multiple linear logistic regression analysis (SAS, Cary, NC); regression coefficients were considered significant at $P < .05$.

**Results**

**Enteric pathogens isolated.** In total, 2254 stool samples from 271 infants and 712 samples from 268 older siblings were
collected. Among the 271 study infants, 72% (195) had ≥8 of the 11 stool samples collected during each infant’s 18-week study period; of the remainder, 9% (24) had 5–7 samples collected and 19% (52) had ≤4 samples collected, and this last group represented infants who dropped out in the first month of the study. Among the siblings, 78% had all 3 stool samples collected; 12% and 10% had only 1 or 2 samples collected, respectively.

Astrovirus was detected in 20% (452/2254) of infant samples and 3% (21/712) of sibling samples. Of 271 study infants, 61% (164) had astrovirus detected in at least 1 stool sample compared with only 7% (7/94) of older siblings. In 30% of infants with astrovirus infection, the virus was detected in only 1 sample; in the remainder, astrovirus was detected intermittently for up to 17 weeks (median, 5). The mean age at study enrollment of infants with ≥1 astrovirus-positive samples was not significantly different from that of infants without astrovirus-positive samples (124 vs. 105 days, P = .1, ANOVA). Rotavirus was detected in 12 stool samples from 10 infants and adenovirus 40/41 in 39 samples from 35 infants. *Salmonella* species were identified in 3 samples from 3 infants, *Shigella* species in 19 samples from 17 infants, and *Campylobacter* species in 40 samples from 35 infants. Twenty infants had concurrent enteric infections with one or more viral or bacterial pathogens (table 1).

During the 18-week period each infant was followed, 31% had none of the six viral or bacterial enteric pathogens isolated, 46% had one, 18% had two, and 5% had three pathogens. No infant had more than three pathogens isolated over the study period. Rotavirus and adenovirus 40/41 were identified in 2 and 6 single stool samples from older siblings, respectively.

**Symptoms reported among study infants.** Over the 18-week period each infant was followed, symptoms reported within 2 days before or on the day of stool collection included 305 reports of diarrhea in 54% (146 infants), 116 reports of fever in 31% (83), 74 reports of vomiting in 16% (43), and 16 reports of anorexia in 6% (15). Annualized symptom rates were 3.25 for diarrhea, 1.24 for fever, 0.79 for vomiting, and 0.17 for anorexia. Of these reported symptoms, single episodes at only one study visit were reported for the majority of infants: 63% of diarrhea (193/305), 79% of fever (92/116), 68% of vomiting (50/74), and 100% of anorexia (16/16) reports. Among those infants with symptoms reported at consecutive visits, 8% of diarrhea episodes were reported at two consecutive 1-week visits, 9% at two consecutive 2-week visits, and 20% at more than two consecutive visits (range, 3–8 weeks; median, 4). The point prevalence of diarrhea per study week among infants varied from 15% to 22% but was not significantly different over the study period (P = .08, χ²). The distribution of diarrhea, fever, vomiting, and anorexia are outlined in table 2.

**Clinical spectrum of astrovirus and other enteric infections.** Detection of astrovirus from stool was significantly associated with concurrent diarrhea but not with other symptoms. Of all 305 diarrhea episodes reported at a visit, 26% (78) were associated with astrovirus; 17% (78/452) of astrovirus-positive infant stool samples were associated with concurrent diarrhea and 9% with other symptoms (astrovirus-positive vs. astrovirus-negative samples, P = .01 for diarrhea, P = .1 for other symptoms, χ²). Rates of adenovirus infection and concurrent symptoms were 18% and 3% for diarrhea or other symptoms, respectively, but neither of these rates was significantly higher among adenovirus-positive versus adenovirus-negative samples. Among the 12 rotavirus-positive samples, 2 (17%) were associated with diarrhea but 5 (42%) were associated with other symptoms, including 4 episodes of concurrent fever and 1 episode of anorexia. No concurrent symptoms were noted among the 3 infants with *Salmonella* species, only 1 of 19 samples with *Shigella* species was associated with a concurrent symptom (fever), and of the 40 *Campylobacter*-positive samples, concurrent symptoms included diarrhea (13%) and fever (5%).

Regression analysis was used to determine whether any factors could be identified to determine risk for any symptoms. The only significant associations found in analysis were between astrovirus infection and diarrhea reported at a study visit (P = .01; odds ratio [OR], 1.44; 95% confidence interval [CI], 1.07–1.92) or consecutive 1-week study visits (P = .04; OR, 1.32; 95% CI, 1.00–1.74). No other symptoms or pathogens were significantly linked by regression analysis.

### Table 1. Single and concurrent enteric infections in 2254 stool samples from 271 infants.

<table>
<thead>
<tr>
<th>Single infections</th>
<th>No.</th>
<th>Multiple infections</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrovirus only</td>
<td>452</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Adenovirus only</td>
<td>28</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Rotavirus only</td>
<td>8</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella</em> only</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Shigella</em> only</td>
<td>16</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><em>Campylobacter</em> only</td>
<td>34</td>
<td>Adenovirus + rotavirus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenovirus + <em>Shigella</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenovirus + <em>Campylobacter</em></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Astro-, aden-, and rotavirus</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. Distribution of single and consecutive reports of symptoms among 271 infants.

<table>
<thead>
<tr>
<th>Symptoms reported</th>
<th>Single visit</th>
<th>Consecutive 1-week visits</th>
<th>Consecutive 2-week visits</th>
<th>&gt;2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea (n = 305)</td>
<td>193 (63)</td>
<td>24 (8)</td>
<td>26 (9)</td>
<td>62 (20)</td>
</tr>
<tr>
<td>Fever (n = 116)</td>
<td>92 (79)</td>
<td>4 (3)</td>
<td>10 (9)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Vomiting (n = 74)</td>
<td>50 (68)</td>
<td>6 (8)</td>
<td>4 (5)</td>
<td>14 (19)</td>
</tr>
<tr>
<td>Anorexia (n = 16)</td>
<td>16 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Seasonal prevalence of astrovirus infections. When the rate of astrovirus-positive samples was adjusted for number of samples obtained per calendar month, significant monthly differences in astrovirus prevalence were identified (figure 1). Because the number of other enteric pathogens isolated was low, seasonal patterns were not distinguished for these other pathogens. Data from the region suggest that the warm, rainy season begins in May and continues through October; however, the village we studied is at the highest elevations, and in that area, the seasons begin at least a month earlier (Collier GA, personal communication). Therefore, significantly increased astrovirus prevalence correlated best with the beginning of the warm, rainy season, whereas significantly decreased astrovirus prevalence correlated best with the cool, dry season.

Discussion

In this prospective study, astrovirus was the most common enteric pathogen isolated from a 3-year birth cohort of rural Mayan infants. Of the infants studied, 61% had astrovirus isolated from at least 1 stool sample over the 18-week period each infant was followed, and astrovirus was associated with almost one-quarter of all diarrheal episodes reported at study visits. Since the seroprevalence of infection in this study was not determined, and overall prevalence was determined solely by EIA detection of pathogens, it was not possible to determine the additional rate of inapparent infections with minimal fecal shedding not detectable by EIA [15] or to determine the rate of primary versus secondary infections.

Infants in this study were found to have the highest astrovirus prevalence reported among infants in any region of the world. The prevalence of astrovirus infection in other studies has varied depending on the nature of the study design. Cross-sectional studies of infants hospitalized with gastroenteritis have identified rates of 2% in India [16], 3% in Brazil [17], and 4.2% in Australia [18]. In cross-sectional studies of infants evaluated for gastroenteritis in the outpatient setting, rates of infection have been higher, ranging from 7.3% among Guatemalan children [19] to 8.6% in Thailand [20]. The highest prevalences have been identified in prospective studies, especially among children attending day care centers, where attack rates as high as 34% have been described [2]. In a prospective Guatemalan study, astrovirus was also the most common enteric pathogen, isolated from 38.6% of children from birth to 3 years of age [19].

In our study, the prevalence of astrovirus was much higher than that of rotavirus (4%) or adenovirus 40/41 (13%). The reason for the high prevalence of astrovirus in this population is unknown but may be related to environmental factors, such as temperature, rainfall, or humidity. An additional finding in this study was a low rate of rotavirus infections, which is known to be affected by environmental factors [21–23]. The geographic region of this study is >2400 m (>8000 feet) above sea level, and the weather is generally cool, with a daily high temperature of 10–16°C (50–60°F), with defined rainy and dry seasons.

Diarrhea was the most prevalent symptom reported in this cohort, with an annualized rate of 3.25 episodes per infant. A review of published studies from 1980 through 1992 demonstrated a global incidence of 2.6 episodes of diarrhea per child per year [24], a rate similar to that demonstrated in our study. In addition, before adjustment for seasonality, the point prevalence of diarrhea per study week did not differ significantly
among study patients, and the age at enrollment of infants did not differ among infants with or without astrovirus detection during the study period. This suggests that diarrheal episodes occurred uniformly in the study population. Fever, vomiting, and anorexia accounted for less than half of all symptoms reported in our study. While the majority of reported symptoms occurred at only one study visit, the recurrence of reported symptoms beyond 2 weeks occurred in up to 20% of infants with diarrhea and vomiting, suggesting the presence of persistent or recurrent enteric infections in a substantial proportion of young infants.

Astrovirus infection was significantly associated with concurrent diarrhea but not with symptoms of vomiting, fever, and anorexia. Prolonged fecal shedding of astroviruses was also reported in this study, with up to 70% of infants intermittently shedding astrovirus for a median of 5 weeks. Despite the significant association of astrovirus with diarrhea, there was a high rate of asymptomatic astrovirus infection, as reported in other studies [2, 19]. The prevalence of astrovirus infection in this study was strikingly age-related and primarily occurred among children in the first year of life. Limited data from seroprevalence studies in developed countries suggest that astrovirus infection occurs early in life but at later ages than demonstrated in our study. For example, a study conducted in England of asymptomatic children and young adults demonstrated that the seroprevalence of astrovirus antibodies was 7% by 6–12 months of age and 75% by 5–10 years of age [25].

The seasonal pattern of astrovirus prevalence was well documented in our study and was consistent with reports from other developing areas of the world, where astrovirus infections have been documented to occur in the winter months or in the rainy season. We found a peak in astrovirus prevalence at the beginning of the warmer rainy season and a significant decrease during the colder dry season. Our study differs from other reports in that the majority of other studies showing a predilection of astrovirus for winter or the rainy season comprised populations living in tropical climates. The environmental conditions in our study area consist of cool temperatures throughout the year, with marked variations in monthly rainfall. Thus, year-round cooler weather may account for the detection of astrovirus throughout the year, with variations in astrovirus prevalence related to rainfall or humidity.

The high rate of astrovirus antigen detection by ELISA methods in this study was confirmed by RT-PCR in a randomly selected subset of ELISA-positive and -negative samples. There was very good concordance as demonstrated by ELISA sensitivity of 84% and specificity of 74% compared with RT-PCR. Thus, a range of astrovirus infection prevalences of 51%–67% was based on sensitivity and specificity of the ELISA in comparison with RT-PCR. The fact that there was not perfect concordance between ELISA and RT-PCR methods is not unexpected, since ELISAs are generally not 100% sensitive or specific. Discordance between ELISA and RT-PCR methods could be due, in part, to the fact that ELISA results indicate presence of both soluble and particle-associated astrovirus antigen while RT-PCR assays detect nucleic acid in intact virus particles. Sample preparation is also more extensive for RT-PCR compared with ELISA and could affect the yield of intact virus available for RT-PCR detection. Finally, the guanidine thiocyanate extraction method for RT-PCR extraction was used to minimize RT-PCR inhibitors but could have sacrificed sensitivity of the assay [26]. Given these limitations, however, the projected range of astrovirus prevalences in this population was 51%–67%, based on correlation of the sensitivity and specificity of ELISA compared with RT-PCR results. These rates are still much higher than those in published studies of other areas of the world.

In summary, our study demonstrated that astrovirus was the most common enteric viral or bacterial pathogen isolated from rural Mayan infants living in impoverished conditions in the highlands of Chiapas, Mexico. Astrovirus infection was significantly more common and significantly associated with diarrhea in infants compared with 2- to 5-year-old siblings. The seasonal pattern of astrovirus infection in this community correlated with increased prevalence in warmer rainy months and decreased prevalence in cooler dry months. There is an increasing recognition of symptomatic astrovirus infection in young infants in this and other studies. However, since the majority of infection is asymptomatic, and since symptomatic illness appears to be mild and self-limited, further investigations should be conducted to determine the impact of astrovirus infection on morbidity and mortality in infants and children, especially among those living in developing areas of the world.

Acknowledgments

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References


