

Prevalence of Astrovirus Infection among Chilean Children with Acute Gastroenteritis

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The frequency of astrovirus infection in 456 Chilean children with diarrhea was determined by enzyme-linked immunosorbent assay, reverse transcriptase PCR, and cell culture. Astrovirus was detected in 16.5% of rotavirus-negative and 7% of rotavirus-positive samples obtained from emergency rooms or hospitals and in 11% of samples from day care centers. HAST-1 was the predominant serotype identified.

The development of simple immunoassays, molecular detection methods, and cell culturing has improved the capability of laboratories to detect astrovirus and has facilitated field studies. Recent studies using these techniques suggest that astrovirus may cause 2 to 8% of diarrheal episodes in children (2, 3, 9, 11). Seven astrovirus serotypes have been described (HAST-1 to HAST-7) (10, 14, 16).

Since 1979, we have been conducting studies to determine the causes of diarrhea in Chilean children. As in most other areas of the world, the majority of diarrheal cases in Chile do not receive an etiologic diagnosis, and test results are usually negative for the commonly recognized bacterial pathogens. Rotavirus is the most common viral agent of gastroenteritis, especially in children under 2 years of age, and other viruses, including astroviruses, may account for many additional cases (5, 7, 21). We were also interested in determining whether astrovirus was more common among children who required medical attention than among children with diarrhea in day care centers (DCCs) and if coinfections occurred commonly in DCC settings.

Three different groups of fecal specimens from children under 3 years of age who presented with acute watery diarrhea were tested for astrovirus. The first group consisted of samples from 292 children who sought care in five emergency rooms (ERs) in Santiago, Chile, between April 1993 and March 1995. These samples are a portion of nearly 2,000 samples obtained during this period as part of a study to characterize the serotypes of rotavirus in circulation (18). We selected 50 to 60 rotavirus-negative samples from each ER that had an adequate volume for astrovirus testing. In addition, we selected 32 rotavirus-positive samples from one ER to estimate the frequency of dual infections.

The second group consisted of 74 stool samples (50 rotavirus negative and 24 rotavirus positive) obtained from children with acute diarrhea admitted to Roberto del Río Hospital between July 1985 and July 1987. These specimens were selected from a total of 256 diarrheal samples collected during this period to study the epidemiological features of rotavirus (1, 6).

The third group consisted of 90 samples obtained between

September 1994 and August 1995 from children with diarrhea who were less than 3 years of age and who attended eight different DCCs in Santiago. These samples represented 75% of all 120 diarrheal episodes detected in these DCCs during a milk-feeding study, and they had been tested previously for the presence of the following pathogens: rotavirus and enteric adenovirus by enzyme-linked immunosorbent assay (ELISA) (12, 20), *Clostridium difficile* toxin by ELISA (Premier *C. difficile* toxin A; Meridian Diagnostics, Inc.), and ova and parasites by direct microscopic observation. Samples were cultured on bacteriological media in order to isolate different enteropathogenic bacteria (4), and strains of *Escherichia coli* were studied with biotinylated DNA probes (8).

All samples were tested for astrovirus by ELISA, as previously described (16, 17). Specimens are typically considered positive if the average absorbance of the positive capture (P) and negative capture (N) wells meet the following criteria: $P/N > 2$ and $P - N \geq 0.07$. For the purpose of this study, we chose a more stringent cutoff ($P - N \geq 0.32$) for positive samples, and we decided to reevaluate nonconclusive samples which included all samples with values between 0.07 and 0.10 (considered indeterminate) and between 0.11 and 0.31 (considered low positives). Samples that fell in the nonconclusive category by the initial ELISA of the fecal extract were further studied by reverse transcriptase PCR (RT-PCR) of RNA isolated directly from the stool and by the inoculation of Caco-2 cell cultures (ATCC HBT37) with fecal filtrates. The cultures were then tested for astrovirus positivity by ELISA of the cell culture supernatant and by RT-PCR of RNA isolated from the cell culture, as previously described (17). ELISA samples strongly positive for astrovirus ($P - N > 1.0$) were serotyped according to the method used by Noel et al. (17).

Astrovirus was detected in 51 (16.5%) of 310 rotavirus-negative samples from children with diarrhea who sought care at ERs or required hospitalization. An additional 28 (9%) samples were nonconclusive by ELISA (Table 1). Detection rates ranged from 8 to 22% and did not correlate with the median age of the children treated at the different ERs. Astrovirus was a coinfecting agent in 7% of the 56 rotavirus-positive samples from diarrheal episodes.

A more complete analysis was done with samples collected in DCCs. One or more pathogens were detected in 58% of 90 samples from diarrheal episodes among children attending DCCs (Table 2). Enteropathogenic *E. coli* (EPEC) (23%) and

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TABLE 1. Astrovirus in stool samples from Chilean children with non-rotavirus-associated or rotavirus-associated gastroenteritis who sought care in ERs or required hospitalization

Rotavirus status and hospital of origin of samples ^a	No. of samples	Median age (mo)	% with ELISA astrovirus result	
			Positive	Nonconclusive ^b
Rotavirus negative ^c				
ER Roberto del Río	60	6	20	13
ER Calvo Mackenna	50	13	22	8
ER Sótero del Río	50	10	18	26
ER E. González Cortés	50	9	10	2
ER Félix Bulnes	50	5	8	0
Hospital Roberto del Río	50	3	20	4
Total	310		16.5	9
Rotavirus positive				
ER Roberto del Río	32	5	6	0
Hospital Roberto del Río	24	3	8	0
Total	56		7	0

^a Samples from ERs were taken from 1993 to 1995; samples from hospitals were taken from 1985 to 1987.

^b Indeterminates and low positives with an optical density differential between 0.07 and 0.31 (see text).

^c Differences in percent positivity among different ERs and between rotavirus-negative and -positive samples were not significant ($P > 0.5$ by the chi-square or two-tailed Fisher's exact test).

enterotoxigenic *E. coli* (ETEC) (14%) were the most common pathogens, followed by astrovirus and *Giardia lamblia*, both detected in 10 (11%) samples from diarrheal episodes. An additional four children had samples that were not conclusive for astrovirus by ELISA, of which two samples were confirmed to be positive, one by cell culture and one by direct PCR. Rotavirus (2%) and enteric adenoviruses (2%) were less common in this population. Half of the astrovirus infections were associated with other pathogens: two with EPEC, one with ETEC, one with EPEC and *G. lamblia*, and one with ETEC and *G. lamblia*. *G. lamblia* was commonly associated with other pathogens. The median ages of children with diarrhea of any etiology and children with astrovirus diarrhea were the same (21 months).

The 32 fecal samples that we considered nonconclusive by the initial astrovirus ELISA were studied by RT-PCR, culture, a second ELISA, and a second RT-PCR (Table 3). The majority of these nonconclusive samples (21 of 32 [66%]) were negative by these methods. Samples initially considered low positive (absorbances of 0.11 to 0.31) as well as samples initially considered indeterminate (absorbances of 0.07 to 0.10) displayed similar profiles with the confirmatory tests. Four samples (13%) were positive by all tests, and seven were positive by at least one method. Possible explanations for the discrepancy in the results are shown in Table 3.

We selected 22 astrovirus isolates for further serotyping. HAst-1 was the most common serotype in both study periods and accounted for 58% of all samples tested. HAst-2 was the next most common (20%), although it was detected only from 1993 to 1995. HAst-4 was detected at low levels in both study periods (8%), and HAst-3, -5, -6, and -7 were not detected.

This study is the first to report astrovirus in fecal specimens from children with diarrhea in Chile and is one of the first to report it in Latin America (2, 13). The prevalence of astrovirus in both rotavirus-negative (16.5%) and rotavirus-positive (7%) stools of Chilean children with diarrhea who sought care in

ERs, were hospitalized, or attended DCCs (11%) is at the upper limit of previous reports of astrovirus in 2 to 8% of all diarrheal episodes in children (3, 11, 16). In Chile, rotavirus is detected in 30 to 40% of children under 3 years of age who seek care for watery diarrhea in ERs (18). Therefore, we estimate that the prevalence of astrovirus-related diarrhea among all children with diarrhea in this age group is close to 10 to 12%, indicating that astrovirus is an important enteric pathogen of children. This figure may be greater (e.g., 15 to 20%) if we consider that of the samples that yielded nonconclusive results by ELISA, approximately one-third (11 of 32) were positive by one or more of the other more sensitive confirmatory methods (i.e., direct RT-PCR or culture in combination with ELISA or RT-PCR).

While ELISA is a simple method for screening fecal specimens for astrovirus, our results indicate that specimens which yield ambiguous results should be confirmed by a different technique, because an additional one-third of the cases (nearly 3 to 4% of all diarrhea cases) were identified with a confirmatory test. A study applying RT-PCR to ELISA-negative stool specimens from children attending DCCs has shown similar rates of detection of astrovirus in children with diarrhea (15). Nevertheless, the advantages of PCR, which can be extremely sensitive, must be assessed further.

The frequencies of astrovirus detection in children with acute diarrhea severe enough to require medical attention and children with milder diarrhea were similar. The high astrovirus detection rates observed in Santiago most probably have been influenced by the age groups selected; other studies have shown that astrovirus infections are more common in children

TABLE 2. Astrovirus and other pathogens isolated from 90 diarrheal episodes that occurred among children attending eight Santiago DCCs

Type of detection	No. of stool samples with pathogens detected		
	Total (%)	As sole pathogen	Associated with one or more viral or bacterial pathogens
Overall pathogen detection			
One or more pathogens	52 (58)	39	13
No pathogens	38	NA ^a	NA
Individual pathogen detection			
Viruses	14	7	7
Astrovirus	10 (11)	5	5
Rotavirus	2 (2)	1	1
Enteric adenoviruses	2 (2)	1	1
Bacteria	42	29	13
EPEC	21 (23)	17	4 ^b
ETEC	13 (14)	8	5
EHEC ^c	6 (7)	3	3
<i>Campylobacter jejuni</i>	1 (1)	1	0
<i>Shigella flexneri</i>	1 (1)	0	1
Parasite	10	3	7
<i>Giardia lamblia</i>	10 (11)	3	7 ^b

^a NA, not applicable.

^b For EPEC detection compared to *G. lamblia* detection, the P value for the difference in isolation as sole versus associated pathogen was <0.01 by two-tailed Fisher's exact test. No significant difference was observed among other pathogens.

^c EHEC, enterohemorrhagic *E. coli*.

TABLE 3. Additional testing of 32 samples with astrovirus-nonconclusive ELISA readings

Result of:			Comment	No. (%) of samples with indicated profile ^a	
Direct RT-PCR	Culture + ELISA	Culture + RT-PCR		Indeterminate	Low positive
–	–	–	Astrovirus negative	13 (68)	8 (62)
+	+	+	Confirmed astrovirus positive	2	2
–	+	+	Possible RT-PCR inhibitors in stools	2	2
+	–	–	Virus not viable?	2	0
+	+	–	Probable technical problem in culture RT-PCR	0	1

^a Samples with an optical density of 0.07 to 0.1 were considered indeterminate; samples with an optical density of 0.11 to 0.31 were considered low positive.

less than 3 years of age (2, 9, 11). In DCCs where a wide variety of other pathogens has been sought, astrovirus was detected in 11% of diarrheal episodes, but in half of these, a second pathogen was also identified. This high rate of mixed infections has been reported previously for astrovirus (15) and may be related to crowding and lack of good hygiene among these children (19). The occurrence of mixed infections did not seem to influence the clinical outcome toward more severe disease, since none of the children in DCCs with single or mixed infections had diarrhea severe enough to require hospitalization.

The distribution of astrovirus serotypes in Chile was comparable to that described for other areas of the world. HAst-1 is the predominant serotype worldwide (17), and HAst-5, -6, and -7 are uncommon.

This report demonstrates that astrovirus is commonly found in fecal specimens from children with acute gastroenteritis in Chile and is the second most commonly detected virus, after rotavirus, among children with diarrhea requiring medical attention. In a preliminary study, enteric adenovirus was detected by ELISA in 4% of these samples (data not published).

Moreover, astrovirus may be more common than rotavirus as a cause of diarrhea among children in DCCs. ELISAs applied directly to stool specimens can effectively detect astrovirus infection in a substantial proportion of children, and confirmatory techniques should be reserved for a small number of specimens whose ELISA results are inconclusive. A prospective study is required to fully understand the epidemiology of astrovirus infection in Chile.

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