

## CHILDHOOD ASTROVIRUS-ASSOCIATED DIARRHEA IN THE AMBULATORY SETTING IN A PUBLIC HOSPITAL IN CORDOBA CITY, ARGENTINA

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### SUMMARY

Human astroviruses have been increasingly identified as important agents of diarrheal disease in children. However, the disease burden of astrovirus infection is still incompletely assessed. This paper reports results on the epidemiological and clinical characteristics of astrovirus-associated diarrhea, as well as the impact of astrovirus infection on the ambulatory setting at a Public Hospital in Córdoba city, Argentina. From February 2001 through January 2002, 97 randomly selected outpatient visits for diarrhea among children < 36 months old were enrolled. A single specimen of stool from each child was collected and tested for astrovirus antigen by enzyme immunoassay. Astroviruses were detected in 12.37% of the diarrheal episodes. All the positive cases occurred in children 4 to 18 months, but the highest rate was in children aged 4 to 6 months (23.80%). The clinical symptoms of astrovirus associated-diarrhea were fever 41.66%, vomiting 25.00% and dehydration 8.33%; overall 16.66% required hospitalization. Astrovirus was identified through the year and no seasonally pattern was detected (cool semester 15.21% versus warm semester 9.80%  $p > 0.05$ ). According to our estimation about one out of seventy-four children in this cohort would be assisted annually for an astroviral-diarrheal episode in the Public Hospital and one out of eight diarrheal cases could be attributed to astrovirus infection. Astrovirus is a common symptomatic infection in pediatric outpatient visits in the public hospital in the study area, contributing 12.37% of the overall morbidity from diarrhea.

**KEYWORDS:** Astrovirus; Infant diarrhea; Outpatient visits; Impact in the medical assistance.

### INTRODUCTION

Since the initial visualization of astrovirus by electron microscopy in 1975, evidence supporting their importance as causes of childhood diarrhea has been slow to accrue<sup>1,10</sup>. Only recently, after improvements in diagnostic methods, has the role of astroviruses as etiological agents of infantile gastroenteritis started to be more appreciated.

The medical importance of human astrovirus infection has been established by reports showing that in some settings astroviruses are second only to rotavirus as a common cause of viral gastroenteritis in infants and young children<sup>6</sup>.

The prevalence of astrovirus infection varied depending on the nature of the study design. In developed countries, this virus has been associated with 4 to 10% of endemic diarrheal episodes in children<sup>2,16</sup>. These data are in agreement with longitudinal studies conducted in Latin America in cohorts of children 0 to 3 years old, which reported that between 5 to 7.3% of the diarrheal episodes in the community were associated with astrovirus<sup>3,7</sup>. Studies of infants hospitalized with acute diarrhea have identified rates between 2 to 5% in India<sup>17</sup>, Brazil<sup>18</sup>, Australia<sup>15</sup>, and Argentina<sup>5</sup>. Although the studies of infants evaluated for gastroenteritis in the ambulatory setting are limited, the rate of

infection has been reported to be higher than in hospitalized children (16.5%)<sup>4</sup>.

The lack of astrovirus diagnostic capability in Argentina results in under-estimation and under-appreciation of the astrovirus impact in the diarrhea-associated disease.

During one year we conducted an astrovirus surveillance among outpatient children under 3 years old who required medical assistance because of acute gastroenteritis illness. We report the epidemiological and clinical characteristics of astrovirus-associated diarrhea, as well as the impact of astrovirus infection as investigated in the ambulatory setting at a Public Hospital in Córdoba city, Argentina.

### MATERIALS AND METHODS

**Study population.** The study was carried out from February 2001 to January 2002 at the Public Pediatric Hospital "Niño Jesús", in Córdoba city, Argentina, which assists approximately 32% of the Cordobezan pediatric population under the public health system (the public health system assists at approximately 55% of the Cordobezan pediatric population). According to the hospital records among the cohort of 0-36 months old, a mean of 2200 episodes are reported annually of acute diarrhea (revised years: 1999 and 2000).

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**Sample selection.** According to the laboratory and physician resources, we decided to enroll for this study approximately 5% of the total diarrhea cases in the cohort of 0 to 36 months old (approximately, one out of twenty diarrhea cases) that fulfilled the following pre-established conditions: 1) they were not referred from other hospital centers; 2) they did not receive any anti microbial therapy; 3) they had experienced less than five days of diarrhea evolution and 4) the stool sample had been collected on the hospital visit day. At enrollment, a pediatrician assessed the degree of dehydration by using World Health Organization criteria based on clinical signs and symptoms<sup>20</sup>, and interviewed the care taken accompanying the children, completed a standardized questionnaire regarding: the sex, date of birth and feeding practice. Only a single specimen of whole stool from each child was obtained and stored at -20° C until tested. Under these conditions, a total of ninety-seven stools were collected.

The patients' mean average age was 12.7 months. The male to female ratio was 1.3.

The authorities of the Hospital were notified about the nature and purpose of the study and their approval was obtained.

**Definitions.** A "diarrheal day" was defined as the occurrence of three or more unformed stools in a 24-hour period.

**Astrovirus detection.** Astrovirus was detected by an enzyme immune assay kit (IDEIA TM Dako diagnostics, Cambridge, United Kingdom). The test combines genus specific monoclonal and polyclonal antibodies which react with and detect known strains of human astrovirus in a solid phase immunoassay. The assay was run according to the manufacturer's instructions.

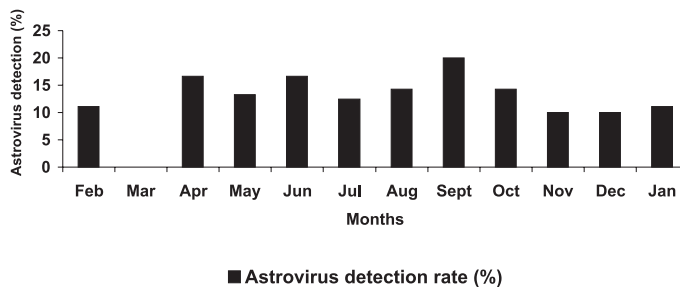
Samples positive for astrovirus antigen were also assayed by enzyme immunoassays for Group A rotavirus antigen (Pathfinder Rotavirus from Sanofi Diagnostic Pasteur, France) and adenovirus 40/41 antigens (Adenocon type 40/41; Cambridge Biotech Corp., Worcester, MA). All the enzyme immunoassays were conducted as specified by the manufacturer's instructions and were read by a spectrophotometer.

**Statistical Methods:** Statistical analysis of the data was made by the Fisher's exact test (two tailed).

## RESULTS

**Epidemiology of Astrovirus diarrhea in the physician visit.** The overall annual prevalence of astrovirus infection was found to be 12.37% (12 of 97 total samples collected); the monthly prevalences of astrovirus detection are shown in Fig 1. Although a slight difference in the seasonal astrovirus pattern was evident (cool semester, from April to September 15.21%, versus warm semester, from October to March, 9.80%), this difference was not significant when analyzed by the Fisher test ( $p > 0.05$ ).

The age distribution of astrovirus infection is shown in Table 1. The mean age of infected children was 8.5 months. All astrovirus infected children were less than 19 months of age. The peak detection rate was observed in children 4 to 6 months old, making up 23.80% of infected children (5/21). This result was statistically significant when it was compared with the rate of infection in the other age groups ( $p < 0.05$ ).



**Fig. 1** - Monthly distribution of astrovirus identified in the stool of children with acute gastroenteritis assisted at the Public Pediatric Hospital "Niño Jesús", Córdoba city, Argentina from February 2001 to January 2002.

**Table 1**

Age distribution of astrovirus infection in children with acute gastroenteritis at the Public Pediatric Hospital "Niño Jesús", Córdoba city, Argentina

Age groups (months)	No. of samples tested	Astrovirus positive samples	
		n	%
0-3	6	0	(0.00)
4-6	21	5	(23.80) *
7-9	14	2	(14.28)
10-12	15	2	(13.33)
13-15	13	2	(15.38)
16-18	10	1	(10.00)
19-21	5	0	(0.00)
22-24	6	0	(0.00)
25-36	7	0	(0.00)
Total	97	12	(12.37)

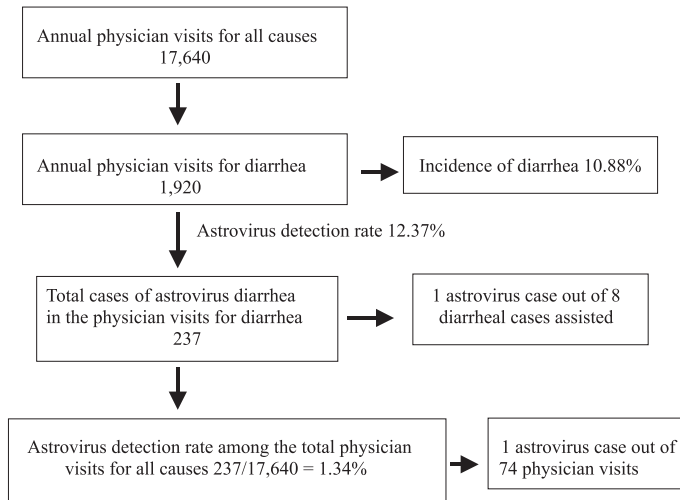
\* Prevalence rate was significantly higher in the age group 4 to 6 months ( $p < 0.05$ )

**Clinical features.** Clinical features of astrovirus infections were documented for the group of 12 pediatric outpatients whose samples were positive. Of these children 83.34% did not require hospitalization, while 16.66% were hospitalized because of gastroenteritis. The mean number of day in the hospital was 1.5 days, range 1 to 2 days. Other clinical features were temperature (measured in the axilla) above 37.8 °C (5/12, 41.66%), vomiting (3/12, 25.00%) and only one case was accompanied by dehydration (1/12, 8.33%). Resolution was good in all patients, although one hospitalized child suffered a dual infection (rotavirus and astrovirus).

**An insight of astroviral diarrheal impact in the physician visits at the Public Pediatric Hospital.** From February 2001 to January 2002, a total of 17,640 children less than 3 years of age visited physicians at this institution. Among this group, 1,920 (10.88%) of the children reported episodes of acute diarrhea (range between 104 to 247 monthly physician visits for diarrhea).

Extrapolations of astrovirus detection rate to the hospital-based data revealed an estimation of physician visits rate for astrovirus gastroenteritis for the whole population less than 36 months old assisted at the Public Pediatric Hospital (Fig. 2). According to these extrapolations about one out of seventy-four children in the cohort of 0-36 months old would be

assisted annually for an astroviral diarrheal episode in the Public Pediatric Hospital and one out eight diarrhea cases in this cohort could be attributed to astrovirus infection.



**Fig. 2** - Annual estimate of the astrovirus diarrheal disease impact on the physician visit in a cohort of children less than 36 months old at the Public Pediatric Hospital "Niño Jesús", Córdoba city, Argentina.

## DISCUSSION

The present study allowed us to explore the age-related prevalence, the seasonality of infection and the medical significance of astrovirus infection from outpatients visits for diarrhea.

The seasonal pattern of astrovirus incidence documented in our study is consistent with a long-term study conducted in Australia<sup>13</sup> in which astrovirus infection shows a pattern without a statistically significant distinct seasonal peak over a period of four consecutive years. In contrast, the majority of other studies describe a predilection of astrovirus for winter or the rainy season in populations living also in temperate regions<sup>3,11</sup>. However, reports exist which describe higher astrovirus prevalences during spring and summer months than during the cold months of the year<sup>7,8,14,19</sup>. The reason of the different seasonal astrovirus pattern is unclear.

The prevalence of astrovirus infection in this study was strikingly age-related and primarily occurred among children in the first year of life. According to our results, the proportional distribution of astroviral infection was as follows: children less or equal 12 months of age 75% (9/12); children between 13 and 18 months old 25% (3/12) and those older or equal 19 months 0% (0/12). Indeed, among the children less or equal 12 months old, this study documents that the highest infection rate was in children 4 to 6 months old (5/9, 55.55%). This may be due in part because according to the records obtained, this age coincide with the time age weaning usually begins (up to 80% of the children aged more than 4 months begin to be fed by animal milk or formula). Also, the maternally derived antibodies might be started to decline. In contrast, almost 92% of the assisted children aged 0-3 months were breastfed milk, and in this group no astrovirus infection was observed. Besides, no infection rates were observed in children older than 18 months. This

observation could be attributed to active immunity to infection that might confer protection to symptomatic or severe infection even in the presence of several co-circulating astrovirus serotypes. The result of seroprevalence studies of antibodies to astroviruses conducted in the United States and the Netherlands are consistent with this notion<sup>9,12</sup>. However, much remains to be learned about protective immunity to astrovirus.

Our report is the first to examine the occurrence of astrovirus in ambulatory patients in Argentina. These results show that the prevalence of astrovirus detection in children with acute diarrhea that required a physician visit was 12.37% and only one sixth (2/12) had to be hospitalized. This suggests astrovirus infection could be related to a mild secretory diarrhea that would not require frequent hospitalization. Nevertheless, it should be taken into account that in a cohort of 0 to 36 months of age, one out of eight diarrhea cases did require medical assistance attributable to astrovirus infection. Moreover, the information that astrovirus was identified only in children < 18 months of age is useful in that expansion of the study could focus on this age group.

Improvements in diagnostic methods have increased the isolation of astrovirus. This discovery provides an insight into the significance of the agent as a pathogen and a causative agent of pediatric gastroenteritis. Understanding the prevalence of different pathogens in gastroenteritis is essential for measuring the magnitude of the public health impact and the effectiveness of intervention strategies.

## RESUMO

### Diarréia associada a astrovírus em crianças de ambulatório em Hospital Público de Córdoba, Argentina

Os astrovírus humanos têm sido identificados como importantes agentes de diarreias em crianças embora o impacto da sua infecção não tenha sido esclarecido. Este estudo não só mostra os resultados das características epidemiológicas e clínicas, mas também o impacto da infecção por astrovírus em pacientes ambulatoriais de um Hospital Público da cidade de Córdoba na Argentina.

Escolheram-se randomicamente 97 pacientes ambulatoriais com menos de 36 meses, entre fevereiro de 2001 e janeiro de 2002, que consultaram por diarreia. Pesquisou-se antígeno de astrovírus por ensaio imuno-enzimático em uma única amostra de fezes por paciente estudado. Determinou-se a presença de astrovírus em 12,37% dos casos de diarreia. Todos os casos positivos foram em crianças de 4 a 18 meses, mas o índice mais elevado se apresentou em crianças de 4 a 6 meses (23,80%). Os sintomas de diarreia associada a astrovírus foram febre 41,66%; vômitos 25,00% e desidratação 8,33%; ou seja, 16,66% dos pacientes precisaram hospitalização.

A presença de astrovírus foi anual sem se observar comportamento sazonal, semestre frio 15,21% versus semestre quente 9,80%  $p > 0,05$ . Em nossa pesquisa, uma de cada 74 crianças seria atendida anualmente por apresentar um episódio de diarreia associada a astrovírus no hospital público e um de cada 8 casos de diarreia poderia atribuir-se à infecção por astrovírus.

Astrovírus é uma infecção sintomática em pacientes pediátricos ambulatoriais, representando 12,37% da morbidade por diarreia.

## ACKNOWLEDGEMENTS

This work received financial support from the Council of Science and Technology of the National University of Cordoba, Argentina (Grant 05/H081/2001-2002). We thank Dr. Roger I. Glass (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) who kindly supplied the astrovirus detection kits.

The authors are grateful to María Rosa Yacci (Department of Statistics, Faculty of Economic Sciences, National University of Cordoba, Argentina) for statistical assistance, and to Dr. Victor O. Giayetto for his Portuguese language assistance

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Received: 23 December 2003

Accepted: 8 March 2004