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ORIGINAL ARTICLE

Astrovirus gastroenteritis in hospitalized children of less than 5 years of age in Taiwan, 2009

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Background/Purpose: Acute gastroenteritis is a common illness in children under 5 years old. Although rotavirus is a leading cause, other viruses including astrovirus are also important, but have been the subject of limited studies. This is a prospective study to investigate astrovirus gastroenteritis in hospitalized children in Taiwan.
Material/Method: From January 2009 to December 2009, children below 5 years of age admitted to three hospitals in Taiwan due to acute gastroenteritis were eligible for this study. Stool specimens were sent for the detection of astrovirus by reverse transcriptase polymerase chain reaction; once positive for astrovirus, the sequencing and phylogenetic analysis of each strain was performed.
Results: A total of 989 children were enrolled during the study period. The overall positive rate of astrovirus was 1.6%, ranging from 1.03% to 2.26% in different hospitals, while rotavirus accounted for 20.2% of the patients. Six of the 16 children (37.5%) with astroviral infection had documented coinfection with rotavirus. The median age of infection was 28.2 months. The seasonal distribution of astrovirus peaked from April to June. Diarrhea alone (40% vs. 2.1%, $p < 0.0001$) was significantly more commonly seen than the triad of fever, vomiting and diarrhea (30% vs. 71%, $p = 0.0062$) in children with astroviral infection alone than in those with rotaviral infection alone. The mean duration of diarrhea was significantly longer in patients with mixed

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infection than those with astroviral infection alone (6.8 vs. 4.2 days, p = 0.013). Respiratory symptoms were noted in 10 children (62.5%). Serotype HAstV-1 was the most common (68.8%).
Conclusion: Astrovirus accounted for 1.6% of infections in children under 5 years hospitalized with acute gastroenteritis in Taiwan. Compared with those caused by rotavirus, the incidence of gastroenteritis in hospitalized children caused by astrovirus was low and the disease severity was mild.

Introduction

Diarrhea is an important cause of childhood morbidity and mortality, especially in developing countries. Astroviruses, which belong to the family *Astroviridae*, are non-enveloped single-stranded positive-sense RNA viruses that were first detected in 1975 by electron microscopy in stool specimens from children with acute gastroenteritis.[1] Human astroviruses have increasingly been identified[2] and are important agents of diarrheal disease, especially in infants and young children.[3] Serologic surveys in the United Kingdom and the United States have shown that over 70% of children acquire astrovirus infection by 5 years of age.[4] Astrovirus infections occur worldwide, and their incidence in children with gastroenteritis in both developing and developed countries ranges from 2% to 9%,[5–10] although some studies have reported a prevalence of up to 26%.[11] Outbreaks of diarrhea due to astrovirus have frequently been reported,[12–16] and have also been associated with nosocomial infections in hospitals.[17,18]

The virus is commonly transmitted via fecal–oral route.[2] The main symptoms of astrovirus infection are similar to rotaviral diarrhea, with watery and mild diarrhea for 2–3 days associated with vomiting, fever, anorexia, abdominal pain and various constitutional symptoms lasting for ≤4 days.[2,19]

Although astroviruses have been commonly been studied using electron microscopy and enzyme immunoassay (EIA) techniques during the past few years, the epidemiological data in Taiwan are limited. This is the first multicenter, prospective study of acute gastroenteritis caused by astrovirus in children below 5 years of age in Taiwan. The aim of this study was to determine the prevalence, age, seasonal distribution and clinical presentations of astrovirus infections among children with acute gastroenteritis in Taiwan.

Materials and methods

Study population

From January 2009 to December 2009 (a 1-year period), children below 5 years of age who were admitted to one of the three participating hospitals due to diarrhea episode were prospectively surveyed. The three hospitals included Chang Gung Memorial Hospital in Linko in northern Taiwan (Hospital A), Changhua Christian Hospital in the middle of Taiwan (Hospital B), and Chang Gung Memorial Hospital in Kaohsiung in southern Taiwan (Hospital C). Children admitted to intensive care units were excluded. Only those with astrovirus infection were included in this study and demographic as were the clinical data from these children that were collected and analyzed. For comparison, data from children with rotavirus infection were also collected.

Diarrhea surveillance

The children from whom stool samples were sent to each hospital's laboratory for microbiological studies during hospitalization were surveyed to determine whether they were experiencing a 'diarrhea episode'. A study nurse or physician visited the ward and reviewed the medical records of these eligible subjects. The patients were enrolled if the clinical symptoms met those of a diarrhea episode and their stool specimens were sent to the central laboratory (Centers for Disease Control, CDC, of Taiwan) for further microbiological tests. The diarrhea episode was defined as watery or loose stool passage more than three times within 24 hours. All stool samples were frozen and stored at −70°C until the time of assay.

Stool collection and microbiological tests

Stool specimens were collected and preliminary screened for rotavirus VP6 antigen using an EIA kit (RIDASCREEN® Rotavirus, R-Biopharm AG, Germany) at each hospital. Specimens were also shipped to the reference laboratory at CDC-Taiwan for further investigation by culture to determine whether *Shigella*, *Campylobacter*, *Salmonella*, *Vibrio* spp., or pathogenic *E. coli* were present. Viruses (rotavirus, norovirus and astrovirus) were tested for using reverse-transcriptase polymerase chain reaction (RT-PCR) methods.

Stool specimens were prepared with phosphate-buffered saline in 10% (wt/v) suspensions clarified by centrifugation (10,000 rpm for 30 minutes). Viral RNA was extracted from 200 μL of clarified suspension by using MagNA Pure Compact Nucleic Acid Isolation Kits (Roche, Germany) according to the manufacturer's instructions. Astrovirus-specific RT-PCR assays incorporating the Mon340/Mon348 primer set were used to amplify a partial ORF1a, as previously described,[20] and other RT-PCR products of partial ORF2 were amplified using primer set Mon269/Mon270,[21] before being determined with an ABI 3130 sequencer (Applied Biosystems, CA, USA). The reference strains included for comparison were as follows:

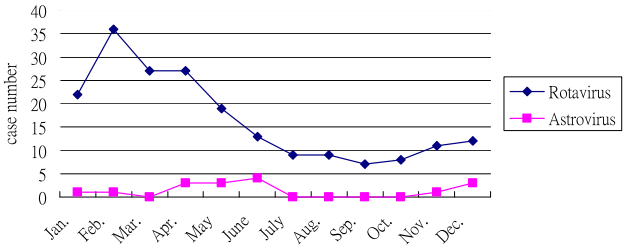

Figure 1. Monthly distribution of rotavirus and astrovirus in children in 2009.

- Human astrovirus type 1 PANSC493/BR/1994(EF535765);
- Human astrovirus type 1 Beijing/176/2006/CHN(FJ755403);
- Human astrovirus serotype 2/US(L13745);
- Human astrovirus type 3/US(AF292074);
- Human astrovirus, serotype 4/Oxford/(L38506);
- Human astrovirus type 5/US(U15136);
- Human astrovirus type 6/Oxford/US(L38507);
- Human astrovirus type 7/US(AF248738); and
- Human astrovirus type 8/US(Z66541).

Multiple nucleotide sequences were aligned and analyzed phylogenetically using MEGA 4.0 software (MEGA, Tempe, AZ, USA).

Statistical analysis

Statistical software SPSS version 10.1.3C (SPSS Inc., Chicago, IL, USA) for Windows was used to record data and analyze results. Chi-squared test was used for comparison of categorical data, and a partitioning of chi-square method (Brunden, 1972) was used for each pair-wise comparison. One-way analysis of variance (ANOVA) with the least significant difference procedure was used in continuous data. Kruskal-Wallis one-way ANOVA by ranks with a multiple-comparison procedure was used with the ordinal data. A p value <0.05 for overall groups was considered statistically significant.

Results

During the 1-year period, a total of 989 cases were enrolled in this study. Of the 989 children, rotavirus was identified in

Table 1 Comparison of clinical features between children hospitalized with rotavirus and astrovirus infections

Characteristics	Rotavirus alone (%)	Astrovirus alone (%)	Mixed (%)	p-value for astrovirus alone and mixed infection	p-value for rotavirus alone and astrovirus alone
Case number	194	10	6		
Age (months)					
Mean ± SD	25.0 ± 14.5	30.2 ± 16.0	26.3 ± 17.0	0.5842	0.2403
Median	21.3	28.2	25.5		
Symptoms					
Diarrhea alone	4 (2.1)	4 (40.0)	1 (16.7)	0.3452	<0.0001
Diarrhea and fever	41 (21.1)	3 (30.0)	3 (49.9)	0.4386	0.5073
Vomiting and diarrhea	11 (5.7)	0 (0.0)	1 (16.7)	0.1967	0.4400
Fever, vomiting and diarrhea	138 (71.1)	3 (30.0)	1 (16.7)	0.5637	0.0062
Bloody stool	29 (15.0)	1 (10.0)	1 (16.7)	0.7055	0.6673
Mucoid stool	77 (39.7)	5 (50.0)	3 (50.0)	1	0.5177
Duration of symptoms (days)					
Vomiting					
Mean ± SD	1.9 ± 2.7	0.7 ± 1.6	0.8 ± 1.6	0.8347	0.0096
>2 days	43 (22.2)	1 (10.0)	1 (16.7)	0.7055	0.3629
Diarrhea					
Mean ± SD	5.5 ± 2.0	4.2 ± 1.4	6.8 ± 1.7	0.0133	0.0698
>5 days	79 (40.7)	1 (10.0)	5 (83.3)	0.0045	0.0529
>8 days	16 (8.3)	0 (0.0)	1 (16.7)	0.1967	0.3453
Fever					
Mean ± SD	3.0 ± 2.4	4.7 ± 6.5	3.2 ± 3.2	0.9300	0.4958
>2 days	99 (51.0)	5 (50.0)	3 (50.0)	1	0.9494
Hospital stay (days)					
Mean ± SD	4.8 ± 2.0	3.5 ± 1.4	3.8 ± 1.3	0.6522	0.0159
>5 days	46 (23.7)	2 (20.0)	1 (16.7)	0.8728	0.7878
Respiratory symptoms (+)	121 (62.4)	7 (70.0)	3 (50.0)	0.4386	0.6274

SD, standard deviation.

200 (20.2%) and astrovirus in 16 (1.6%) cases. Six children were positive for both rotavirus and astrovirus. Most of astrovirus infections were identified during April and June (Fig. 1). The positive rate of astrovirus infection in the three hospitals was 2.26% (10/442) in Hospital A, 1.18% (3/255) in Hospital B and 1.03% (3/292) in Hospital C, respectively.

Table 1 illustrates the clinical features of the children with astrovirus, rotavirus and mixed infection. Respiratory symptoms were noted in 10 cases (62.5%) of astrovirus infection. For the 10 children with astrovirus infection alone, the median age was 28.2 months. Forty per cent of them had diarrhea alone and 30% had the triad of fever, vomiting and diarrhea. The mean durations of fever and diarrhea were 4.7 days and 4.2 days, respectively. The mean hospital stay was 3.5 days. Half of the children had mucoid stool and 10% had bloody stool.

The children with mixed infection had a significantly longer duration of diarrhea (6.8 ± 1.7 days vs. 4.2 ± 1.4 days, $p = 0.0133$) and a larger proportion had diarrhea for >5 days (83% vs. 10%, $p = 0.0045$) than those with astrovirus infection alone. Compared with those with rotaviral infections, children with astroviral infection alone had a significantly higher rate of diarrhea only (40% vs. 2.1%, $p < 0.0001$), and had a significantly lower rate of the triad of fever, vomiting and diarrhea (30% vs. 71%, $p = 0.0062$). The incidence of bloody or mucoid stool was comparable between these two groups. There was no significant difference between the two groups in terms of the duration of fever, vomiting or diarrhea, and hospital stay. Neither were there significant differences the laboratory data, including white blood cell (WBC) counts, C-reactive protein (CRP) levels and aspartate aminotransferase (AST) levels, of children with astroviral or rotaviral infections (Table 2).

The history of vomiting or diarrhea within 1 week among household members was identified in 39.7% of children positive for rotavirus alone and in 20% of those positive for astrovirus alone ($p = 0.2137$).

All 16 astrovirus strains were further typed based on a partial ORF1a region. Phylogenetic analysis revealed that 11 (68.8%) of the 16 strains were clustered in Human astrovirus type 1 (HAstV-1), one strain (6.2%) in Human astrovirus type 2, one strain (6.2%) in Human astrovirus type 5 and the remaining three (18.8%) strains were un-typeable (Fig. 2). The partial ORF2 nucleotide sequences were deposited in GenBank with accession numbers JF343966 to FF343978. These 11 HAstV-1 cases could be divided into two clusters. Cluster I had a nucleotide sequence that was 95.4%–96.8% identical to a Brazilian strain (GenBank accession number EF535765) and cluster II were 99.1–99.7% identical to a Chinese strain (GenBank accession number FJ755403).

Discussion

Results from the present study showed that astrovirus accounted for 1.6% of children under 5 years of age who

Table 2 Comparison of laboratory data on admission between the children with rotavirus infection and those with astrovirus infection

Laboratory data	Rotavirus alone ($n = 194$) (%)	Astrovirus alone ($n = 10$) (%)	Mixed ($n = 6$) (%)	*p*-value for astrovirus alone and mixed infection	*p*-value for rotavirus alone and astrovirus alone
Hb (g/dL)					
Mean ± SD	12.6 ± 1.3	12.7 ± 1.1	12.5 ± 0.8	0.7448	0.7042
No. of data missing	1	0	0		
Leukocyte (1000/μL)					
Mean ± SD	11.6 ± 5.3	10.0 ± 3.7	9.0 ± 3.6	0.7202	0.3532
<5	3 (1.6)	0 (0.0)	0 (0.0)	—	0.6919
>15	38 (19.7)	2 (20.0)	1 (16.7)	0.8728	0.9808
No. of data missing	1	0	0		
Platelet (1000/μL)					
Mean ± SD	313.3 ± 91.8	353.9 ± 95.5	270.7 ± 73.9	0.1226	0.2307
<150	2 (1.0)	0 (0)	0 (0)	—	0.7469
No. of data missing	1	0	0		
CRP (mg/L)					
Mean ± SD	19.9 ± 38.4	10.8 ± 9.1	30.1 ± 30.9	0.5868	0.6958
>10	64 (40.3)	3 (42.9)	3 (60.0)	0.5751	0.8910
>20	40 (25.2)	1 (14.3)	2 (40.0)	0.3315	0.5152
>40	21 (13.2)	0 (0)	2 (40.0)	0.0793	0.3050
No. of data missing	35	3	1		
AST (U/L)					
Mean ± SD	48.2 ± 21.4	49.0 ± 14.7	68.5 ± 29.0	0.6326	0.9765
>50	18 (28.6)	1 (33.3)	1 (50.0)	0.7389	0.8598
No. of data missing	131	7	4		

AST, aspartate transaminase; CRP, C-reactive protein; Hb, hemoglobin; SD, standard deviation.

Figure 2. Phylogenetic dendrogram of astrovirus sequences yielded from acute gastroenteritis in children from Taiwan in 2009. The phylogenetic tree was constructed based on partial ORF2 nucleotide sequences. Scale bars are proportional to the phylogenetic distance.

were hospitalized with acute gastroenteritis in Taiwan. This rate is lower than that of previous reports from other countries, giving rates of 2% to 9.7%.[5–10,17,22–27] Several explanations may be given for the low prevalence in the present study. First, only inpatients were included in this study and the patients who needed hospitalization were supposed to have more severe symptoms. Most previous reports[5,7,26] included outpatients and indicated that children with astrovirus infections had a lower score of disease severity, so the results from the present study may underestimate the role of astrovirus in children under 5 years of age with acute gastroenteritis in Taiwan. Second, though the overall prevalence rate of 1.6% was relatively low, the incidence rate of 2.26% in Hospital A situated in northern Taiwan was comparable with that from previous reports.[5–10] The geographic difference in climate among the three hospitals in Taiwan may influence the prevalence of astrovirus infection in the study population. Third, due to the relatively small sample size, the quality and the timing of the specimens collected from the patients may affect the results.

Previous reports[17,26,27] have indicated that the highest incidence rate of astrovirus infection occurred in infants of less than 12 months, which is younger than found in the present study (most infections occurred during the second year of life). The peak incidence of astrovirus in the current study was from April to June, which was similar to that in the United States.[27] The children with astroviral infections had a significantly lower rate of the presentation of the triad of fever, vomiting and diarrhea compared with the children with rotaviral infection, but a higher rate of diarrhea only. These observations may suggest lower disease severity in children with astrovirus than rotavirus infection, though the duration of fever, vomiting, diarrhea and hospital stay were comparable. The proportion of children with diarrhea more than five days was higher in those with rotavirus than astrovirus infection (40.7% vs. 10%), but was not statistically significant. There was no significant difference between the two groups in terms of laboratory data, including WBC counts, serum CRP levels and AST levels.

More than half of the children with astrovirus infections had respiratory symptoms. The previous reports from Japan[28] and Taipei[26] had similar findings, with cough and rhinorrhea being evident in children with acute astrovirus infection. This finding indicates that astrovirus infection is not only an isolated gastrointestinal infection, but may be a systemic infection. Further studies are needed to elucidate this issue.

Eight serotypes of human astroviruses have been recognized, denoted HAstV-1 to HAstV-8. All eight HAstV serotypes or genotypes have been detected throughout the world.[21,29,30] HAstV-1 appears to be the most common based on both serotypic[13] and genotypic surveys. In our

study, HAstV-1 was the most common serotype, which was a similar result to that found in a previous study in Taipei.[26] Seroprevalence studies in the United States and elsewhere have shown that more than 90% of children have antibodies to HastV-1 by the age of 9 years,[13] suggesting that infection, although largely asymptomatic, is common.

There were several limitations in the present study. First, the study period was only 1 year and the number of astrovirus-positive cases was too small. Second, only inpatients were included and those who were sent to the emergency room or outpatient clinic were excluded, resulting in the exclusion of potential positive cases with mild disease severity. Third, though we collected clinical manifestations we did not record the severity score for each case. Further studies should include disease severity.

The present study was the first multicenter, prospective study of astrovirus gastroenteritis in children in Taiwan and it provides information about the prevalence and span of clinical spectra associated with astrovirus infections. The current study showed that infection with astroviruses may be an important cause of respiratory symptoms, as well as gastroenteritis, in children. Further epidemiological 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.

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