Development of Two-phase and Pellet method for concentration of Rotavirus and its application to recovery of Rotaviruses from sewage
Despite considerable advances in current public health services and hygiene control in water supply, sanitation and wastewater treatments, diarrhea still remains a potential risk to human health, especially in infants and young children, in both developed and developing countries. Epidemiological studies have proven that diarrheal diseases (17%) in addition to acute respiratory infections (17%), are the most common causes of death among children aged less than 5 years of age around the world in 2004. The role of norovirus in pediatric gastroenteritis is increasingly appreciated, as a recent study demonstrated that noroviruses are the second most frequent causative agents of viral diarrhea in young children. Current estimates of annual global childhood mortality associated with norovirus infection is up to 200000 deaths. Studies conducted in different countries have shown that enteric adenoviruses are responsible for 2% to 23% of the cases of acute diarrhea among infants and young children. Astrovirus causes sporadic cases of acute diarrhea in young children. The importance of viral pathogens in children with acute gastroenteritis in the south of Iran

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BACKGROUND AND OBJECTIVES: Different types of viruses are the leading cause of acute diarrhea among infants and young children worldwide. Epidemiological surveillance of viral agents is critical for the development of effective preventive measures, including vaccines. This study aimed to determine the prevalence of the four major enteropathogenic viruses—rotavirus, norovirus, adenovirus and astrovirus—in children over 7 years of age.

DESIGN AND SETTING: A cross-sectional descriptive study conducted on stool specimens of children with acute gastroenteritis admitted to the Pediatrics Unit of 17 Shahrivar Hospital in Borazjan, Iran from October 2008 to September 2010.

PATIENTS AND METHODS: Acute gastroenteritis was defined as ≥3 loose watery stools per 24 hours. A total of 375 stool samples were collected from hospitalized children aged <7 years old with acute gastroenteritis. All samples were investigated by using enzyme-linked immunosorbent assay for the presence of viral antigens.

RESULTS: Rotavirus was detected in 91 (24.3%) of the patients whereas the prevalence of norovirus, adenovirus and astrovirus was 12.5%, 5.1% and 2.4%, respectively. On average, 75.9% of children with viral diarrhea were younger than 2 years old (P=.023). All the strains of viral gastroenteritis studied peaked in the autumn, except for adenovirus which peaked in spring (P=.015). The most common clinical symptoms included diarrhea (92.2%), vomiting (68.7%), abdominal cramp (60.8%) and moderate dehydration (57.2%).

CONCLUSION: Since nearly half of gastroenteritis cases (44.3%) were due to viral agents, testing for the viral antigens may guide the clinical approach to those patients with acute diarrhea particularly in the case of children less than 2 years old, and during cold seasons.
radic cases and small outbreaks of gastroenteritis, responsible for 2% to 16% of infections among hospitalized children with diarrhea.\textsuperscript{14-16} Despite the importance of these enteric virus infections in child morbidity and mortality rates in developing countries, there is no specific surveillance system for viral gastroenteritis in Iran, and laboratory diagnosis is rarely performed. To date, very few studies have investigated the prevalence of viral agents among hospitalized Iranian children with acute gastroenteritis.\textsuperscript{16-18} Moreover, to our knowledge, this is the first study that has compiled complete information correlating the clinical features and etiological role of the different viruses in children with acute diarrhea in Iran.

The present study aimed to determine the prevalence of four important diarrheagenic viruses (rotavirus, norovirus, enteric adenovirus, and astrovirus) in children aged <7 years old who were hospitalized for acute gastroenteritis in Borazjan, Iran. In addition, we examined the age and seasonal distribution as well as clinical symptoms associated with viral agents in this population.

**PATIENTS AND METHODS**

From October 2008 to September 2010, a cross-sectional descriptive study was conducted on 375 stool samples collected from hospitalized children less than 7 years old, suffering from acute gastroenteritis, in 17 Shahrivar Hospital in Borazjan, Iran. Acute gastroenteritis was defined as ≥3 loose watery stools per 24 hours. All the fecal specimens were sent to the virology laboratory of Bushehr University of Medical Science and stored at −70°C until the time of assay. All samples underwent only one cycle of thawing and freezing prior to characterization. Demographic (age and sex) and clinical data (days of hospitalization, diarrhea, vomiting, fever, convulsion, abdominal cramp and severity of dehydration) and also the type of feeding (as breast/bottle feeding) that were useful for our investigation were gathered for each case by using a standard structured questionnaire. According to WHO recommendations, all children presenting with gastroenteritis were classified in specific age groups (e.g., 0-2, 3-5, 6-8, 9-11, 12-17, 18-23, 24-35, 36-47, 48-60 and 61-83 months) so that age-specific incidence rates of hospitalization could be calculated.\textsuperscript{19}

All fecal specimens were examined for the presence of four enteric viruses: rotavirus, norovirus, adenovirus and astrovirus. These viruses were detected by commercially available enzyme immunoassay (EIA) kits according to manufacturer’s instructions (Rotavirus/Adenovirus/Astrovirus Antigen, Generic Assays kit, Dahlewitz, Germany) and IDEIA Norovirus EIA (Oxoid, Ely, United Kingdom). The tests detected specific antigens for group A rotaviruses, enteric adenoviruses, astrovirus and norovirus, respectively.

Data was statistically analyzed by SPSS version 17 (SPSS Inc., Chicago, IL, USA). Statistical analysis by the chi-square test was used to analyze the data obtained to the age group, sex and seasonal distribution of the viral agents. The Fisher exact test was used to analyze the clinical symptoms. A P value <.05 was considered statistically significant.

**RESULTS**

Among the 375 stool specimens collected from children with acute diarrhea, 166 (44.3%) contained at least one of the four viruses. In particular, all viral infections were mono-infections and no cases of mixed viral infections were detected. Overall, group A rotavirus was the pathogen most frequently detected (24.27%) (91/375), followed by norovirus (12.53%) (47/375), enteric adenovirus (5.1%) (19/375) and astrovirus (2.4%) (9/375).

Association between age group and isolated viral enteric pathogens are shown in Table 1. All the patients with acute diarrhea ranged between the ages of 1 and 83 months, and the median age was 22 months. Children less than 24 months of age accounted for 75.9% (128 cases) of the overall viral infections, with those between 12 and 17 months of age being the most affected (28.3%) (47 cases) (P=.023). Only one virus (rotavirus) was detected in the age group of 0-2 months. The prevalence of viruses was low in infants aged 0-8 months, peaked in infants aged 9-17 months and declined again among children of 18-83 months of age.

According to the seasonal distribution, it was observed that viral infections were detected with higher frequency in the cold seasons of the year (P=.015) (Table 2). The highest prevalence of rotavirus (55.0%) (50/91), norovirus (63.8%) (30/47) and astrovirus (55.6%) (5/9) infections were identified in autumn (median incidence of 53.6%), while enteric adenovirus peaked in spring (63.2%) (12/19). The lowest incidence of all viral-positive episodes was reported in the summer (median incidence of 3.6%).

**Figure 1** shows the monthly distribution of viral agents in patients with acute diarrhea. The highest rate of detection of rotavirus gastroenteritis was found between the months of November and February (median rate of 85.7%) and the lowest in September, in which no infection was detected (P=.991). Adenoviruses were detected continuously in a 5-month period lasting from February to June with the most frequent between April and May (P=.002). Most of the norovirus-positive cas-
Table 1. Age distribution of viral agents in patients with acute gastroenteritis.

<table>
<thead>
<tr>
<th>Age groups (month)</th>
<th>Rotavirus group (n=91)</th>
<th>Norovirus group (n=47)</th>
<th>Adenovirus group (n=19)</th>
<th>Astrovirus group (n=9)</th>
<th>Global positive (n=166)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>3 (3.30%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (1.81%)</td>
</tr>
<tr>
<td>3-5</td>
<td>10 (10.99%)</td>
<td>7 (14.89%)</td>
<td>3 (15.79%)</td>
<td>1 (11.11%)</td>
<td>18 (10.84%)</td>
</tr>
<tr>
<td>6-8</td>
<td>9 (9.89%)</td>
<td>5 (10.64%)</td>
<td>3 (15.79%)</td>
<td>2 (22.22%)</td>
<td>17 (10.24%)</td>
</tr>
<tr>
<td>9-11</td>
<td>14 (15.38%)</td>
<td>9 (19.15%)</td>
<td>3 (15.79%)</td>
<td>1 (11.11%)</td>
<td>28 (16.87%)</td>
</tr>
<tr>
<td>12-17</td>
<td>26 (28.57%)</td>
<td>14 (29.78%)</td>
<td>4 (21.05%)</td>
<td>3 (33.33%)</td>
<td>47 (28.31%)</td>
</tr>
<tr>
<td>18-23</td>
<td>8 (8.79%)</td>
<td>3 (6.38%)</td>
<td>3 (15.79%)</td>
<td>-</td>
<td>15 (9.04%)</td>
</tr>
<tr>
<td>24-35</td>
<td>8 (8.79%)</td>
<td>4 (8.52%)</td>
<td>2 (10.53%)</td>
<td>1 (11.11%)</td>
<td>14 (8.43%)</td>
</tr>
<tr>
<td>36-47</td>
<td>5 (5.49%)</td>
<td>2 (4.25%)</td>
<td>-</td>
<td>1 (11.11%)</td>
<td>8 (4.82%)</td>
</tr>
<tr>
<td>48-60</td>
<td>5 (5.49%)</td>
<td>2 (4.25%)</td>
<td>2 (10.53%)</td>
<td>1 (11.11%)</td>
<td>10 (6.02%)</td>
</tr>
<tr>
<td>61-83</td>
<td>3 (3.30%)</td>
<td>1 (2.13%)</td>
<td>2 (10.53%)</td>
<td>-</td>
<td>6 (3.61%)</td>
</tr>
</tbody>
</table>

Table 2. Seasonal distribution of viral agents in patients with acute gastroenteritis.

<table>
<thead>
<tr>
<th>Season</th>
<th>Rotavirus group (n=91)</th>
<th>Norovirus group (n=47)</th>
<th>Adenovirus group (n=19)</th>
<th>Astrovirus group (n=9)</th>
<th>Global positive (n=166)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>6 (6.59%)</td>
<td>6 (12.77%)</td>
<td>12 (63.16%)</td>
<td>2 (22.22%)</td>
<td>26 (15.67%)</td>
</tr>
<tr>
<td>Summer</td>
<td>2 (2.20%)</td>
<td>3 (6.38%)</td>
<td>1 (5.26%)</td>
<td>-</td>
<td>6 (3.61%)</td>
</tr>
<tr>
<td>Autumn</td>
<td>50 (54.95%)</td>
<td>30 (63.83%)</td>
<td>4 (21.05%)</td>
<td>5 (55.56%)</td>
<td>89 (53.61%)</td>
</tr>
<tr>
<td>Winter</td>
<td>33 (38.26%)</td>
<td>8 (17.02%)</td>
<td>2 (10.53%)</td>
<td>2 (22.22%)</td>
<td>45 (27.11%)</td>
</tr>
</tbody>
</table>

Figure 1. Monthly distribution of viral agents in patients with acute gastroenteritis.

es occurred from October to February, with a peak of positivity detected in November (40.4%) \((P= .031)\). The highest prevalence of astrovirus was found in November (44.4%). The presence of astrovirus remained low from June to October in which no virus was detected \((P=.183)\).

Clinical characteristics of children infected with different types of viral agents are shown in Table 3. The survey of clinical symptoms showed that diarrhea, vomiting and abdominal cramps were present in all viral-infection cases. Convulsion was only found among patients infected with enteric adenovirus (5.3%). No fever was present in astrovirus infection cases. Among detected manifestations, diarrhea was the most predominant. This was present in all 9 patients who were positive for astrovirus, in 83 of 91 (91.2%) of the patients positive for rotavirus, in 45 of 47 (95.7%) of the children infected with norovirus and in 16 of 19 (84.2%) patients positive for adenovirus. In terms of severity of dehydration, moderate (median rate of 57.2%) was the most common type among all of the viruses detected, followed by severe (26.5%) and mild (16.3%) dehydration, respectively.

DISCUSSION

Pediatric acute gastroenteritis remains one of the most common disorders in infants and young children. This is a leading cause of admission to hospital in industrialized countries and a major source of childhood morbidity and mortality in developing countries. Among enteric viruses, four major categories have been recognized...
as the most significant etiological agents of acute gastroenteritis among children worldwide, namely group A rotavirus, norovirus, adenovirus and astrovirus.3,7,20

In the present study, we assessed the prevalence of these enteric viruses, mentioned above, in stool samples of 375 children aged less than 7 years old who were hospitalized for acute gastroenteritis in the south of Iran. To our knowledge, this is the first such report of an extended study of viral agents in hospitalized children with acute diarrhea in Iran over 24 months. The rate of virus-positive stool samples (44.3%) was in agreement with what was reported in previous studies on hospitalized children.8,9,12,14 The most common virus in the current study was group A rotavirus, which has been associated with 24.3% of infants and children under 5 years of age. This result was consistent with other previous studies worldwide that show rotaviruses as the most important cause of gastroenteritis requiring hospitalization in 15% to 40% of all cases of diarrhea in children.7-9,17,21,22 Therefore, policies to decrease acute gastroenteritis in infants and young children should best be focused on prevention of group A rotavirus infections. The only known adequate primary preventive measure against rotavirus disease is the development of immunization and vaccination programs for children at high risk.

Norovirus has been recently recognized as the second most common enteropathogenic virus among children with acute gastroenteritis in France, Poland,8 Japan,9 and Korea.33 Our study confirmed this data in Iranian children, as noroviruses were identified as the second virus present in 12.5% of the evaluated samples. The proportion of adenovirus (5.1%) reported in this study is in keeping with studies conducted in France,7 Poland,8 Venezuela,9 Japan,15 China,24 and lower than that reported in Korea (7.1%),23 Turkey (10.5%),12 and the Netherlands (23%).13 In the current study, the incidence of astrovirus infection (2.4%) was comparable to what was previously reported in Iran and in other studies.7,8,12 In the present study, mixed infections involving two or more viruses were not detected. This finding is in disagreement with studies conducted in other countries.7,8,13,23

The occurrence of the viral agents that were cumulatively observed in the first 24 months of life (75.9%) is more than in the older age groups, as was found in previous investigations.4,7-9,12,14 The high frequency of viral pathogens in this age group highlights the need for a vaccine to offer optimal protection against these viruses, especially rotavirus as the most significant causes of gastroenteritis, requiring hospitalization in children <2 years old.

Extensive studies in different regions of the world have shown that in temperate climates, rotavirus infection occurs predominantly during the cooler months.25,26 On the other hand, seasonal patterns in tropical climates have reported rates of rotavirus diarrhea throughout the year with seasonal trends that are less pronounced.26-28 Our study showed that there was a significant correlation between the seasonal distribution and rotavirus-positive cases. Rotavirus gastroenteritis occurred throughout the year, with more cases occurring in the winter with a seasonal peak observed in the months of November and February. These findings are similar to those reported in countries with temperate climates such as Spain,29 China,30 and Iran.31 Similar surveillance over a prolonged time period are needed in order to ascertain accurately the seasonality

**Table 3. Distribution of clinical symptoms associated with viral agents in patients with acute gastroenteritis.**

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Rotavirus group (n=91)</th>
<th>Norovirus group (n=47)</th>
<th>Adenovirus group (n=19)</th>
<th>Astrovirus group (n=9)</th>
<th>Global positive (n=166)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>83 (91.21%)</td>
<td>45 (95.74%)</td>
<td>16 (84.21%)</td>
<td>9 (100%)</td>
<td>153 (92.17%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>59 (64.83%)</td>
<td>41 (87.23%)</td>
<td>8 (42.10%)</td>
<td>6 (66.67%)</td>
<td>114 (68.67%)</td>
</tr>
<tr>
<td>Fever</td>
<td>49 (53.85%)</td>
<td>27 (57.45%)</td>
<td>11 (57.89%)</td>
<td>-</td>
<td>87 (52.41%)</td>
</tr>
<tr>
<td>Convulsion</td>
<td>-</td>
<td>-</td>
<td>1 (5.26%)</td>
<td>-</td>
<td>1 (0.60%)</td>
</tr>
<tr>
<td>Abdominal cramp</td>
<td>51 (56.04%)</td>
<td>39 (82.98%)</td>
<td>6 (31.58%)</td>
<td>5 (55.56%)</td>
<td>101 (60.84%)</td>
</tr>
<tr>
<td>Dehydration severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe dehydration</td>
<td>22 (24.18%)</td>
<td>15 (31.91%)</td>
<td>4 (21.05%)</td>
<td>3 (33.33%)</td>
<td>44 (26.51%)</td>
</tr>
<tr>
<td>Moderate dehydration</td>
<td>52 (57.14%)</td>
<td>26 (55.32%)</td>
<td>12 (63.16%)</td>
<td>5 (55.56%)</td>
<td>95 (57.23%)</td>
</tr>
<tr>
<td>Mild dehydration</td>
<td>17 (18.68%)</td>
<td>6 (12.77%)</td>
<td>3 (15.79%)</td>
<td>1 (11.11%)</td>
<td>27 (16.26%)</td>
</tr>
</tbody>
</table>

as the most significant etiological agents of acute gastroenteritis among children worldwide, namely group A rotavirus, norovirus, adenovirus and astrovirus.3,7,20
associated with rotavirus infection in the area of study.

In the current study, adenovirus infection was detected throughout the year. Most cases of adenovirus gastroenteritis occurred in the spring and winter with a seasonal peak observed in the months of April through May. These findings are in keeping with studies conducted in other countries. The highest prevalence of norovirus and astrovirus infections were found in the autumn and winter, as previously described from other countries.

During this study, diarrhea and vomiting which are associated with viral pathogens were the symptoms most commonly reported. These findings are in keeping with studies conducted in Iran, and other countries.

Because of having some limitations in sensitivity and specificity, EIA is not a reliable method for detection of pathogens in samples. In recent years, molecular methods have rapidly found their way into virological studies. Generally, different studies have considered PCRs as specific and sensitive techniques for detection of enteric viruses, such as norovirus and rotavirus, in samples. For this reason, we suggest the use of PCR methods for assessing the presence of viral particles in future surveillance studies. One limitation of the current study is that the true viral infection prevalence could be higher than estimated (44.3%) because we evaluated only hospitalized children with moderate to severe gastroenteritis and the proportion of viral etiological agents among children with only home care or outpatient visits has not been estimated. Another possible limitation is that our results in this study may not be representative of the prevalence of enteric viruses among all 0- to 7-year-old Iranian children. Since we identified the prevalence of viral gastroenteritis from only one city in Iran, to have a comprehensive picture of disease burden of enteric viruses and the distribution and frequency of rotavirus strains circulating to recommend the vaccine in the country, it is necessary to continue the same surveillances in other regions of Iran.

In conclusion, the results of the present study emphasize the importance of detection of enteric viral pathogens, especially rotavirus in stools of hospitalized children with acute gastroenteritis. That suggests that such systematic virological controls at the time of admission could be of major interest in preventing the nosocomial transmission of viral gastrointestinal infections in pediatric departments. The high frequency of the disease burden of rotavirus, provides strong support to the concept that the development of immunization and vaccination programs of rotavirus may have a major impact in reducing rotavirus gastroenteritis morbidity and the pressure on healthcare services due to acute diarrhea, particularly among children less than 2 years old in Iran.
GASTROENTERITIS IN IRAN

REFERENCES
