Epidemiology of Acute Viral Gastroenteritis in Children Hospitalized in Rouen, France

Aude Marie-Cardine,1 Karine Gourlain,2 Olivier Mouterde,4 Nathalie Castignolles,2 Marie-France Hellot,3 Eric Mallet,1 and Claudine Buffet-Janvresse2

Departments of 1Medical Pediatrics, 2Virology, and 3Biostatistics, Rouen University Hospital, France; and 4Department of Medical Pediatrics, Sherbrooke University Hospital, Quebec, Canada

This study assessed the epidemiologic characteristics of acute viral gastroenteritis in hospitalized children. A stool sample obtained from each child was analyzed for the presence of astrovirus, calicivirus, rotavirus, adenovirus, enterovirus, and digestive bacteria. Of the 438 stool samples obtained, 138 tested positive for ≥1 pathogen during the winters of 1997–1998 and 1998–1999 (P<.001). Virologic tests revealed rotavirus in 17.3% of samples, calicivirus in 7.3%, astrovirus in 6.8%, adenovirus in 0.7%, and ≥1 virus in 5.4%. Median age was higher for patients with rotavirus gastroenteritis than it was for those with astrovirus or calicivirus gastroenteritis (P = .014). Mean duration of hospitalization was statistically significantly lower for children with rotavirus gastroenteritis (P = .022), despite the more-frequent dehydration observed among children with rotavirus versus those with astrovirus or calicivirus gastroenteritis (P = .007). In contrast, enteral rehydration was more rapidly achieved in patients with gastroenteritis due to rotavirus.

In France, the epidemiologic characteristics of acute viral gastroenteritis are more easily identified in non-hospitalized adults and children than they are in hospitalized patients. For the nonhospitalized population, the epidemiologic characteristics are detected by the sentinel surveillance of general practitioners, known as the Sentinelle system [1, 2]. This system collects data on the morbidity only of patients treated in cities. It does not specifically indicate the types and the frequencies of the viruses responsible for gastroenteritis [3]. Analyses of stool samples are routinely performed only in 2% of cases. It is well established that rotavirus infection mainly affects children aged ≤2 years [4]. In fact, there is no clinically valid reason to attempt to identify the etiologic agent of gastroenteritis, because, to date, no efficient antiviral drug or vaccine against rotavirus has been developed [5, 6]. In all cases, treatment consists of hydration.

In addition to the data from the Sentinelle system, other studies have reported epidemics of acute viral gastroenteritis in children treated in cities [4, 7]. The epidemiology of nosocomial diarrhea has recently been reported for hospitalized children [8, 9]. The exact epidemiology of acute viral gastroenteritis in hospitalized children remains unknown [10]. Moreover, to our knowledge, no study has reported complete information regarding the number and distribution of viruses responsible for gastroenteritis (rotavirus, astrovirus, calicivirus, and adenovirus) in hospitalized children, although some data do exist concerning only 1 or 2 of these viruses [7, 10–15]. Viral and epidemiologic data from hospitals would be useful to help explain the reasons for hospitalization of children who have acute viral gastroenteritis.

We present the results of what is, to our knowledge, the first reported study conducted involving hospitalized children (age, 1–35 months) with acute viral gas-
Table 1. Number of children with acute viral gastroenteritis who were or were not hospitalized, according to age.

<table>
<thead>
<tr>
<th>Age, months</th>
<th>Treated at home</th>
<th>Hospitalized*</th>
<th>All</th>
<th>Infected with a virusb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>—</td>
<td>19</td>
<td>3 (16)</td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>107</td>
<td>138</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td>7–12</td>
<td>118</td>
<td>148</td>
<td>59 (40)</td>
<td></td>
</tr>
<tr>
<td>13–18</td>
<td>96</td>
<td>58</td>
<td>21 (36)</td>
<td></td>
</tr>
<tr>
<td>19–24</td>
<td>61</td>
<td>36</td>
<td>10 (28)</td>
<td></td>
</tr>
<tr>
<td>25–30</td>
<td>45</td>
<td>15</td>
<td>5 (33)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>37</td>
<td>24</td>
<td>5 (21)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>464</td>
<td>438</td>
<td>138 (32)</td>
<td></td>
</tr>
</tbody>
</table>

* Three adenovirus and 24 dual infections are not reported here.

b The most stool samples were obtained from children aged 1–12 months; the highest percentage of virus-positive stool samples occurred among patients aged 12–18 months (P = .007). Values in parentheses are percentages of children infected with a virus among all those hospitalized.

Gastroenteritis, excluding children with nosocomial and bacterial gastroenteritis. The study period encompassed 2 successive winter seasons (1997–1998 and 1998–1999); there was an epidemic of viral gastroenteritis during the second winter, but not during the first. The prevalence and distribution of adenovirus, astrovirus, calicivirus (Norwalk-like viruses and Sapporo-like viruses), enterovirus, and rotavirus were investigated in both the epidemic and nonepidemic periods by reverse-transcriptase PCR (RT-PCR) and EIA [16–25]. The epidemiologic characteristics of the hospitalized children (i.e., distribution and number of the viruses isolated, exact role of astroviruses and caliciviruses, presence of dehydration, age of children, duration of hospital stay) and their relationship to the different viruses were evaluated.

PATIENTS, MATERIALS, AND METHODS

Patients. The study was performed during 2 winter periods (December 1997 through February 1998 and December 1998 through February 1999); there was an epidemic of acute viral gastroenteritis during the latter period. Children who presented to the hospital were enrolled in the study; they were aged 1 month to 35 months. The study was conducted in 2 medical pediatric wards of Rouen University Hospital, France. Rouen is located in the region of Seine-Maritime, which has a population of ~1 million people. Ethical approval was obtained.
Figure 2. Number of rotavirus, adenovirus, astrovirus, and calicivirus infections in cases of acute viral gastroenteritis. Each bar represents the proportion of infections with 1 of the 3 predominant viruses in monoinfections or in dual infections for each winter season. Dual infections were more common during the second winter period than they were during the first. According to the percentage, rotavirus was more often associated with calicivirus than was astrovirus. Association between rotavirus and astrovirus had the same frequency during both winters.

Methods. Severity of clinical dehydration was defined by the presence of skinfold, tachycardia, and weight loss of 5%–10% of the normal weight. The severity of dehydration was determined by biological tests (i.e., determination of the serum creatinine level, hematocrit, alkaline reserve, and natriemia). Clinical evaluation included a search for signs of lower respiratory tract disease (e.g., bronchiolitis) and other extraintestinal conditions, as well as family hygiene status. In France, poor hygiene is associated with prolonged hospitalization.

Stool samples. Stool samples were obtained only once from each child during the first 48 h of hospitalization. All samples were stored at 4°C until they underwent further analysis. Aliquots of specimens were later stored at −80°C. The presence of rotavirus and adenovirus was determined by use of the SlideX Rota Kit 2 (bioMérieux) and Adenolex (Orion Diagnostics), respectively. Astrovirus was detected with an EIA kit (IDEIA Astrovirus; Dako Diagnostics). Culture for enterovirus was performed as follows: an extract of the sample was cultured on MRC5 in minimal essential medium (pH, 7.2–7.4) with 5% fetal bovine serum (BioWhittaker; Dako Diagnostics) and penicillin (25 U/mL), streptomycin (10 μg/mL), and amphotericin B (0.25 μg/mL). Cultures were incubated at 37°C for 10 days before the stool samples were determined to be negative for enterovirus. Cytopathic effect consistent with enterovirus was confirmed by polyvalent fluorescent antibody staining, performed according to the manufacturer’s instructions (IMAGEN Enterovirus; Dako Diagnostics).

RT-PCR was performed as follows: total RNA was purified from 200 μL of stool extract, prepared in sterile distilled water with an RNA purification kit (Bioprobe Systems) according to the manufacturer’s instructions. The RNA was suspended in a final volume of 25 μL of diethyl pyrocarbonate–treated water. RT-PCR for the detection of astrovirus was performed with use of primers described by Mitchell et al. [26]. Five astrovirus serotypes were grown in tissue culture and were detected by
use of an EIA kit; the threshold of detection was 5–15 ng of viral protein. The sensitivity and specificity of EIA results were 90% and 93%, respectively, compared with the RNA probe hybridization method. The results of the 2 tests correlated well [26].

Calicivirus (Norwalk-like and Sapporo-like viruses) were investigated by RT-PCR with use of primer sets. For Norwalk-like viruses, we used the association of RT-PCR and Southern blot hybridization tests with internal nucleotide probes, on the basis of techniques described elsewhere [27–30]. To validate the RT-PCR results, control stool specimens obtained from persons without diarrhea were tested. Bacteriologic data regarding Salmonella species, Shigella species, Campylobacter species, and Yersinia enterocolitica were obtained for each stool sample.

Statistical analysis. Analysis was performed by use of the Mann-Whitney test or the Kruskal-Wallis test, for continuous factors, and either Pearson’s $x^2$ test or Fisher’s exact test, for discontinuous factors, depending on sample size. The Kruskal-Wallis test was performed according to Dunn’s procedure [31]. When the Kruskal-Wallis test revealed a significant difference, the Bonferroni correction was used to compare 2 groups. The Bonferroni correction consisted of multiplying the $P$ value by the number of comparisons. Only the $P$-adjusted values are reported here. Analyses were performed using StatView, version 5.0 (SAS Institute), and StatXact, version 3.1 (Cytel Software). $P < .05$ was considered to be statistically significant.

RESULTS

Of the 902 children who presented to the pediatric emergency department, 438 were hospitalized in the 2 medical wards. The study took place during 2 consecutive winter seasons (December 1997 through February 1998 and December 1998 through February 1999). For each child, a single stool sample was obtained. The distribution of children according to age is shown in table 1. All children had diarrhea 2–3 days before their hospitalization and all were initially examined by either a pediatrician or a general practitioner located in the city. A total of 438 stool samples were analyzed, and etiologic viral agents were identified in 165 children (37%), including 101 girls (61%) and 64 boys (39%). The mean and median ages were 11 and 9 months, respectively (range, 3 weeks to 35 months). Mean and median durations of hospitalization were 5.5 and 5 days, respectively (range, 1–18 days). In 10 cases, the patient’s poor hygiene resulted in prolonged hospitalization. In 10 other cases, the duration of hospitalization was longer than necessary because enteral nutrition was delayed.

Laboratory investigation revealed 3 predominant viruses: rotavirus, astrovirus, and calicivirus. Ninety-four (57%) of the 165 culture-positive samples yielded rotavirus; rotavirus was responsible for 76 monoinfections and 18 dual infections. Astrovirus was observed in 45 cases (27%; 30 monoinfections and 15 dual infections). Calicivirus was found in 43 cases (26%; 32 monoinfections and 11 dual infections). The distribution of the different viruses is presented in figure 1. Figure 2 shows the distribution of each viral cause of gastroenteritis in monoinfected and coinfected children. There were 6 and 18 cases of dual-infection gastroenteritis during the winters of 1997–1998 and 1998–1999, respectively.

In 1998–1999, astrovirus was detected in 45 cases by use of PCR methods and in 25 cases by use of EIA. The use of sensitive RT-PCR and 4 primer pairs of each sample and Southern hybridization with internal oligonucleotides probes showed a clear predominance of Norwalk-like viruses belonging to the ge-

Table 2. Infecting virus and relationship to demographic characteristics of 438 children with acute viral gastroenteritis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type of infection</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rotavirus</td>
<td>Astrovirus</td>
</tr>
<tr>
<td>No. (%) of children infected</td>
<td>76 (17.4)</td>
<td>30 (6.8)</td>
</tr>
<tr>
<td>Age, weeks$^a$</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Median</td>
<td>40–150</td>
<td>4–140</td>
</tr>
<tr>
<td>Range</td>
<td>12–150</td>
<td>4–140</td>
</tr>
<tr>
<td>Length of hospital stay, days$^b$</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Range</td>
<td>1–13</td>
<td>2–15</td>
</tr>
<tr>
<td>No. (%) of dehydrated children$^c$</td>
<td>43 (57)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Percentage of children with otitis</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Percentage of children with bronchiolitis</td>
<td>19</td>
<td>33</td>
</tr>
</tbody>
</table>

$^a$ A significant difference was observed between rotavirus, astrovirus, and calicivirus infections ($P = .016$).

$^b$ A significant difference was observed between rotavirus and astrovirus infections ($P = .022$).

$^c$ Dehydration occurred significantly more frequently among children with gastroenteritis due to rotavirus than it did among children with gastroenteritis due to astrovirus or calicivirus ($P = .022$).
Figure 3. Number of dehydrated and nondehydrated children, by age and infecting virus. The number of dehydrated children with rotavirus gastroenteritis was higher than the number with gastroenteritis due to other viruses ($P = .007$). No cases of gastroenteritis due to rotavirus were detected in infants aged 0–1 month. Astrovirus was more frequently identified in infants aged 1–12 months (17 children without dehydration and 8 children with dehydration), but the number of dehydrated patients did not increase. Calicivirus was detected in patients both with and without dehydration, regardless of age (23 patients without dehydration and 89 children with dehydration).

notype 2 strain (31 of genotype 2 vs. 5 of genotype 1) during this period [32, 33]. The total number of adenovirus isolates recovered was low ($n = 8$). With regard to the other pathogenic agents, gastroenteritis was associated with enterovirus infection in 11 cases and with Salmonella infection in 3 cases.

Table 2 shows the clinical characteristics of the children in the study (e.g., age, duration of hospitalization, presence of dehydration, and clinical signs), according to the type of infection—rotavirus, astrovirus, calicivirus, or dual infection. Children who presented with gastroenteritis due to rotavirus were older than those with gastroenteritis due to astrovirus ($P = .016$). The age range was lower in patients with gastroenteritis due to calicivirus and astrovirus than it was for those with rotavirus gastroenteritis. Most children with gastroenteritis due to rotavirus and calicivirus were aged 1–18 months (81% and 84%, respectively). Duration of hospitalization for patients with gastroenteritis due to astrovirus was statistically significantly longer than that for patients with gastroenteritis due to rotavirus or calicivirus ($P = .022$). In all cases, the duration of hospitalization was not longer for patients with a lower respiratory tract disease ($P = .060$).

The percentage of patients who had dehydration was higher among those with gastroenteritis due to rotavirus (57%) than it was among those with gastroenteritis due to calicivirus (28%) or astrovirus (30%; $P = .007$; table 2). We studied the number of dehydrated and nondehydrated children with regard to their age and the type of infecting virus (figure 3). Biological data and the findings of clinical examinations showed that dehydrated infants had worse symptoms when rotavirus was responsible for the gastroenteritis. Figure 3 shows that the risk of dehydration associated with rotavirus was higher in patients aged 7–18 months, compared with the risk associated with astrovirus and calicivirus. Risk of dehydration in patients with astrovirus and calicivirus gastroenteritis decreased rapidly after the ages of 13 and 18 months, respectively. Table 3 indicates that the duration of the hospital stay was not correlated with the presence of dehydration. Table 2 shows that the presence of bronchiolitis or otitis did not increase the severity of gastroenteritis, regardless of the virus concerned.

**DISCUSSION**

To our knowledge, this is the first study to have evaluated the epidemiologic characteristics of hospitalized children with acute...
viral gastroenteritis during 2 different winter seasons, one an epidemic period and the other not. The results for 165 virus-infected stool samples were analyzed. The number of stool samples positive for virus was low compared with the number reported in other studies [4, 34–36]. These hospitalized children represent a small percentage of infected children. Acute viral gastroenteritis is generally treated by the general practitioner or family pediatrician, and, therefore, only a small percentage of infected children are hospitalized [1]. All children included in our study were hospitalized for a few days after the onset of viral gastroenteritis.

As reported by other investigators [4], viral excretion was minimal and could not be detected using routine biological tests. However, the use of more sensitive methods could possibly increase the detection rates for calicivirus and rotavirus [37, 38]. Health care providers do not routinely test for astrovirus and calicivirus in France or in other countries [39–44]. In our study, the number of patients infected with astrovirus, calicivirus, and rotavirus could have been underestimated because of the intermittent excretion of viruses in the stool. The low percentage of viruses noted in stool (37%) was not the result of problems in methodology, because we noted the presence of astrovirus, which commonly affects children. Moreover, we did not include cases of nosocomial gastroenteritis in our study. Rotavirus, astrovirus, and calicivirus are known nosocomial viruses in children and adults [20, 45, 46].

In epidemic and nonepidemic periods, the predominant viral agent of gastroenteritis is rotavirus, particularly in hospitalized children aged 6–18 months. Rotavirus is the major etiologic agent of diarrhea in young children in both developed and undeveloped countries [13]. In Rouen, we noted the same incidence of rotavirus during 2 successive winters. In comparison, during the same period, a rotavirus epidemic occurred in Paris [47, 48].

The national epidemiologic characteristics of acute viral gastroenteritis in infants during the winter of 1998–1999 differed from those of the previous winter. Calicivirus and astrovirus were the 2 viruses implicated in the gastroenteritis epidemic. Calicivirus infections were less common among children than they were among adults [49]. The RT-PCR method was found to be useful for the detection of calicivirus. Furthermore, this method was used to assess samples obtained from infants during both the first and the second winter, in which the incidences of calicivirus were not similar.

Calicivirus is a causal viral agent that has been implicated in minor epidemics or viral gastroenteritis [17, 50, 51]. One important finding of our study is the importance of calicivirus in gastroenteritis in hospitalized children. Before our study, the exact role played by calicivirus in hospitalized children was poorly known. The possibility of calicivirus being transmitted to children at their homes was reported by Chiba et al. [52]. Unlike Chiba et al. [52], we did not determine that any sporadic cases of calicivirus had occurred [53–56]. In fact, the prevalence of calicivirus was probably underestimated, as demonstrated in a major study conducted in England in 1990–1995 [57]. The means of transmission varied: transmission often occurred via food, person-to-person contact, or the water supply [32, 58]. As reported by other authors, we also found a predominance of genotype 2 calicivirus [15, 56, 59–62]. Genotype 2 strains have been observed in young children with gastroenteritis in different countries, and a high prevalence of antibodies to the Mexico strains (i.e., genotype 2 strains) was reported in London [63].

Astrovirus can be identified in 5%–20% of cases of acute viral gastroenteritis [64–67]. A study conducted by the Seine Aval program during 4 consecutive years confirmed the presence of astrovirus in the Seine River before the year that the viral gastroenteritis epidemic occurred in Rouen [68, 69]. Furthermore, the presence of adenovirus in the Seine River was also found over several consecutive years. Moreover, a major study, which was performed over a 12-year period, indicated

<table>
<thead>
<tr>
<th>Age, months</th>
<th>Rotavirus Dehydrated</th>
<th>Rotavirus Nondehydrated</th>
<th>Astrovirus Dehydrated</th>
<th>Astrovirus Nondehydrated</th>
<th>Calicivirus Dehydrated</th>
<th>Calicivirus Nondehydrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2–6</td>
<td>5.5</td>
<td>6</td>
<td>7.5</td>
<td>5</td>
<td>9.5</td>
<td>6</td>
</tr>
<tr>
<td>7–12</td>
<td>5.5</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>13–18</td>
<td>4.5</td>
<td>4</td>
<td>0</td>
<td>5.5</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>19–24</td>
<td>4.5</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>10.5</td>
</tr>
<tr>
<td>25–30</td>
<td>4</td>
<td>2.5</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&gt;30</td>
<td>3.5</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

NOTE. Duration of hospitalization was longer for patients who had gastroenteritis due to astrovirus, regardless of whether they were dehydrated, than it was for those with gastroenteritis due to other viruses ($P = .022$). A higher median duration of hospital stay was observed for patients who had gastroenteritis due to astrovirus.
that the prevalence of adenovirus varied from year to year [70].
An epidemic period (1998–1999) explains the high number of
dual infections we saw during the second winter of our study.
Astrovirus was often present in patients with dual infections
[26]. Dual viral infections were the same in a large study, which
found that a majority of rotavirus infections were combined
with adenovirus, calicivirus, or astrovirus [4].

In the majority of patients with gastroenteritis caused by
rotavirus, dehydration was detected during a clinical exami-
nation and confirmed by biological testing. Regardless of the
child’s age, the patient’s condition stabilized with intravenous
rehydration. Enteral nutrition was well tolerated and was begun
24 h after the start of the intravenous perfusion. This may
explain the short period of hospitalization, as compared with
patients who had gastroenteritis due to astrovirus. With regard
to calicivirus gastroenteritis, dehydration is less common and
less severe than is the dehydration associated with rotavirus
gastroenteritis; however, the mean durations of hospitalization
are the same. In fact, like astrovirus infection, treatment for
dehydration was required for a longer time in patients with
calicivirus gastroenteritis than it was for patients with rotavirus
gastroenteritis. There was no selection bias in our study, because
all children were consecutively selected using the same methods.
The presence of lower respiratory tract disease did not prolong
the patients’ hospital stay.

The higher mean age of children with gastroenteritis due to
rotavirus noted in our study was similar to that noted in studies
from other countries; for example, in Guatemala, a major study
reported that the mean age of children infected with rotavirus
was 9 months [71]. The median age was lower for patients who
had gastroenteritis due to astrovirus or calicivirus than it was for
those who had gastroenteritis due to rotavirus. In fact, children
were infected with astrovirus and calicivirus during the first year
of life, whereas rotavirus was found during the first 2 years of
life [46]. Some authors suggest that maternal antibodies against
rotavirus offer better protection to infants [72, 73], which could
possibly explain the low incidence of rotavirus infection in infants
aged 0–6 months. It could be useful to determine whether the
severity of dehydration due to rotavirus gastroenteritis differs
according to serotype. A recent study has reported that a modi-
fication has been observed in the rotavirus serotype common
among the population of Paris [48].

In conclusion, in the majority of cases, the children with
acute viral gastroenteritis who required hospitalization were
infected with rotavirus. Furthermore, calicivirus infection must
be considered when there is a period of epidemic gastroenteritis.
Routine testing for virus should be proposed for patients during
the critical period of infection to determine the exact roles of
calicivirus and astrovirus infection; this virus testing will also
help determine the role these viruses play in nosocomial di-
arrhea. Nosocomial diarrhea extends the length of hospital stay
and consequently increases the cost of hospitalization. More-
ever, children with invasive diarrhea should be given antibiotic
treatment when infection with Salmonella species or Escherichia
coli O157:H7 is suspected. In some cases, calicivirus and as-
rovirus are not detected, and this could also prolong hospi-
talization [74]. Furthermore, a better knowledge of the epi-
demiology of acute viral gastroenteritis would be valuable for
producing an effective vaccine.

Acknowledgments

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