Prevalence of Rotavirus, Adenovirus, Norovirus and Astrovirus Infections and Co-infections Among Hospitalized Children in Northern France

Short title
Viral gastroenteritis among hospitalized children in France

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Abstract: (206 words)

From January to December 2007, 973 stool specimens were prospectively collected from children hospitalized for gastroenteritis signs or from neonates and premature cases who were born in two French hospital settings in the north of France. They were tested by rapid EIA analyses for rotavirus and adenovirus and by two commercially available ELISA tests for the detection of norovirus and astrovirus. The overall rates of prevalence for rotavirus, norovirus, adenovirus and astrovirus were 21, 13, 5 and 1.8%, respectively, and they did not significantly differ between the two hospital settings ($P=0.12$). Mixed virus infections were detected in 32 (3.3%) of the 973 study children and were associated with norovirus in 21 (66%) infants, including 5 premature cases. From falls to spring, norovirus infections accounted for 52% of documented gastroenteritis viral infections at a time where rotavirus was epidemic, resulting in mixed norovirus and rotavirus gastrointestinal tract infections. Of the 367 documented viral gastroenteritis cases, 15 (4.1%) were identified as nosocomial infections 5 of which occurred in premature cases. These findings highlight the need to implement norovirus and astrovirus ELISA detection assays in association with rapid EIA rotavirus and adenovirus detection assays for the clinical diagnosis and the nosocomial prevention of gastroenteritis viral infections in pediatric departments.

Key words: virus, gastroenteritis, multiple infections, hospitalized children
Acute gastroenteritis is a common disorder in young children and the associated dehydration is a leading cause of admission to hospital in industrialized countries and a major source of mortality in developing countries (15). Enteric viruses have been recognized as the most significant etiologic agents of the disease and four categories of viruses are being considered as clinically relevant: group A rotavirus (family Reoviridae), norovirus (family Caliciviridae), adenovirus 40/41 (subgenus F) and astrovirus (3,8,11). In children, group A rotavirus (RV) is the major etiologic agent of viral gastroenteritis and is responsible for 29 to 45% of hospitalizations worldwide (13,14). Recent work has showed that noroviruses are the second most frequent etiologic agents of viral gastroenteritis in children (6,7). Data on the relative significance of the other agents as pediatric pathogens depend on variants such as the diagnostic assay or the geographical setting chosen (9,12,18,19).

Currently, rotavirus, norovirus, astrovirus and adenovirus 40/41 have been recognized as the most significant etiological agents of childhood viral gastroenteritis in industrialized countries. The importance of these four viral agents as a cause of gastroenteritis outbreaks is well documented, but their role in sporadic acute severe gastroenteritis responsible for hospitalization or nosocomial infections remains to be assessed in developed countries (7,8). The aim of this study was to determine the prevalence and the seasonal distribution of the aforementioned viruses in clinically relevant (i.e., hospitalization-requiring) infantile gastroenteritis and to assess the prevalence of nosocomial infections. The third objective was to determine the distribution of mono- and co-infections between these viral agents responsible for gastroenteritis.

From January to December 2007, 973 consecutive fecal specimens documented to be free of common bacterial pathogens were prospectively collected from children (n=859; mean age=1.9, ± SD 2.41 years) who were admitted to one of two hospital settings implicated into the present clinical study (Reims Medical University Center or Brest Medical University...
Center, Northern of France) for classical gastroenteritis signs and from a second patient group consisting of neonates (n=85; mean age=8 ± SD 12 days) and premature infants (n=29; mean aged: 55± SD 356 days) who had never left the hospital and who developed clinical signs of gastroenteritis during their hospitalization stage (13,15). For each of the stool sample taken from these children, rotavirus and adenovirus were detected prospectively by “Rapid EIA assays” (r-Biopharm, Saint-Didier au Mont D’Or, France) following the manufacturer’s recommendations. Norovirus and astrovirus were detected retrospectively by commercially available ELISA tests for the detection of viral antigens in frozen stool samples following the manufacturer’s recommendations (Ridascreen, r-Biopharm). All the experiments were performed in each hospital settings (Brest and Reims) under the same conditions following the manufacturer’s recommendations for the rapid EAI assays (r-Biopharm, Saint-Didier au Mont D’Or, France) and for the ELISA assays (Ridascreen, r-Biopharm). A nosocomial infection by gastroenteritis viruses was defined as the development of classic gastrointestinal signs occurring in children 96 hours after the time of a previous hospitalization or in neonates or premature cases who were born at the hospital (2). For statistical analyses, the nonparametric Mann-Whitney U test and the chi-square or Fischer’s exact tests were carried out when necessary with SAS software, version 8.2 (SAS Institute, Cary, NC, USA). Results were considered as statistically significant for two-sided $P$-values <0.05.

The two hospital cohorts appeared to be non-significantly different in age distribution (2.22 ± SD 3.77 years vs. 1.59 ± 2.48 years; $P$=0.12) or in the number of admissions over the 2007 study period ($P$=0.24). The overall rates of prevalence for rotavirus, norovirus, adenovirus and astrovirus were 21, 13, 5 and 1.8%, respectively, and they did not significantly differ between the two hospital cohorts ($P$=0.12). Single infection cases were detected in 335 (34%) of the 973 study children whereas mixed virus infections were detected in 32 (3.3%) of the same study children. Norovirus was associated in 21 of 32 (66%) mixed
infections cases, including 5 premature cases (Figure 1-A). The most frequent dual gastrointestinal infections were rotavirus and norovirus (50% of 32), adenovirus and rotavirus (16%), rotavirus and astrovirus (13% of 32), norovirus and adenovirus (9% of 32) and norovirus and astrovirus (3%). Surprisingly, we also detected 3 cases of triple gastrointestinal tract infections, 2 of which occurred in two premature children, that were not associated with disease severity criteria such as dehydration or longer duration of hospitalization (not shown). Multiple infections appeared to be more significantly detected during autumnal periods when norovirus was epidemic ($P<0.001$). We observed that the number of rotavirus infections appeared to be significantly higher from March through May ($P<0.001$) and that norovirus and adenovirus infections were more frequently detected during fall and winter months, respectively ($P=0.016$ and $P=0.002$) in hospitalized children. During winter months, norovirus infections accounted for 52% of documented gastroenteritis viral infections at a time when rotavirus was also epidemic, resulting in 16 mixed norovirus and rotavirus gastrointestinal tract infections (Figure 1-B). The number of norovirus positive cases in children aged 0-24 months appeared to be significantly higher than that observed in children aged 25-28 months ($P=0.022$); no other significant variation of frequency was observed for the other viral parameters between the age groups (Figure 2). No norovirus or gastrointestinal virus-associated deaths were reported.

Of the 367 documented viral gastroenteritis cases, 15 (4.1%) were identified as nosocomial infections: 5 (5/15, 33%) occurred in premature infants, 5 (33%) in neonates hospitalized for acute bronchiolitis, 3 (20%) in neonates with cancer undergoing chemotherapy treatment and 2 (14%) in neonates who had never left the hospital. Three of the 5 nosocomial infections occurring in premature infants were identified as dual gastrointestinal tract infections and were associated with a classic clinical syndrome (Table 1). Of the 9 detected norovirus nosocomial infections, 3 (33%) occurred in premature infants (Table 1).
In the present study, we assessed the prevalence of rotavirus, norovirus, astrovirus and adenovirus 40/41 in stools samples collected from children hospitalized for gastroenteritis and from neonates and premature infants who have never left the hospital. We combined rapid EIA assays for rotavirus and adenovirus antigen detection to classical ELISA assays for the detection of norovirus and astrovirus antigens in stool samples of 973 children hospitalized for acute gastroenteritis during a 12 month-period in the north of France. To our knowledge, this is the first extended report of viral infection and co-infections in hospitalized children in France over 12 months. As previously described worldwide in hospitalized pediatric cases, we observed a predominance of rotavirus infections followed by norovirus and lower rates of adenovirus and astrovirus infections (Figure 1) (7-11,17). However, we did not investigate the presence of other recognized enteric viruses such as sapoviruses, toroviruses, kobuviruses or aichi viruses for which molecular detection would probably have significantly increased the overall proportion of documented viral gastroenteritis cases (4).

In the present study, norovirus infection was more prevalent in children in aged 0-24 months ($P=0.022$) and appeared as the second most frequent cause of acute severe sporadic gastroenteritis requiring hospitalization, as previously described in Europe (figure 2) (7,8). We observed that the mean duration of a hospital stay was not significantly longer in norovirus-positive than in norovirus-negative children and those neonates and premature infants who have never left the hospital ($P>0.5$). However, further prospective multi-centric studies are necessary to assess the clinical impact and the severity of norovirus gastrointestinal infection by age classes specifically in neonates and premature infants.

In the present study, mixed infections involving two or more viruses were present in 3.3% of tested samples corresponding to approximately 10% of positive viral detection in stool samples (Figure 1). Norovirus, adenovirus and astroviruses were the most frequently detected viruses implicated in mixed infections in association with rotavirus (figure 1).
Previously published studies identified mixed infection in percentages ranging from 4.4 to 29% of the pathogen-positive stool samples in different European countries using classic or molecular techniques (8,10,11,16). A recent French study reported the presence of 11% of mixed infections among the positive stool samples during 1997 and 1998 winter periods (10).

As reported in this study, no specific clinical severity of the gastroenteritis was significantly related to the detection of mixed infection diseases. Moreover, we observed that mixed infections were no more frequently detected in a specific children age group ($P=0.17$) (Figure 2). However, the presence of mixed viral infections has previously been associated with the development of necrotizing enterocolitis in some premature infants (1,5). This previously published clinical data, in association with our epidemiological results, emphasize the clinical importance of the detection of mixed infections as a cause of severe diarrhea in hospitalized children in Europe and more specifically in those less than 12 months of age.

In our investigation, a nosocomial source of infection was demonstrated in 15 (4.1%) of 367 documented viral gastroenteritis infections (Table 1). No specific clustering of nosocomial infections was identified; the origin of these infections was a chronic viral excretion in the stools of the nurses. The present testing was useful to identify the infected infants as well to detect the chronic intestinal shedding in the paramedical staff. A previous investigation concerning the detection of rotavirus in hospitalized children over 10 years reported that premature neonates made up 26% of the nosocomial gastroenteritis infections and with unexpected high mortality levels of 0.1% (2). In the present study, norovirus was detected in 12 of the 15 documented cases of nosocomial gastrointestinal infections occurring in 5 cases in premature babies (Table 1). Our data highlight the need for norovirus detection in stools of hospitalized children and, more specifically, of premature cases, suggesting that such systematic virological control at the time of admission could be of major interest to prevent the nosocomial transmission of gastroenteritis viruses in pediatric departments.
In conclusion, our findings provide evidence that noroviruses can be a leading cause of viral gastrointestinal infections, and highlight the need to implement norovirus and astrovirus ELISA detection assays in association with rapid rotavirus and adenovirus EIA detection assays for the clinical diagnosis and nosocomial prevention of viral gastrointestinal infections in pediatric departments.

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References:


Mixed Infections
N=32 (32/973= 3.2%)
Adenovirus
12%
Norovirus
32%
Astrovirus
5%
Rotavirus
51%

Figure 1: Distribution (A) and seasonality (B) of Rotavirus, Norovirus, Astrovirus and Adenovirus Positive Antigenic Detection by EIA or ELISA Assays among 973 Stool Samples of Hospitalized Children in Northern France

Single Infections
N=335 (335/973= 34%)

Rota+Noro
50%
Rota+Astro
13%
Noro+Adeno
9%
Noro+Astro
3%
Noro+Rota+Adeno
6%
Rota+Adeno+Astro
3%
Adeno+Rota
16%

Mixed Infections
N=32 (32/973= 3.2%)

Rota+Noro
50%
Noro+Rota+Adeno
6%
Noro+Astro
3%
Noro+Adeno
9%
Rota+Astro
13%
Adeno+Rota
16%

Total number of tested children (n) (57) (49) (70) (76) (85) (58) (52) (88) (111) (166)
Table 1. Virological, demographic and clinical characteristics of the 15 cases of nosocomial infections identified among the 367 documented viral gastrointestinal infections

<table>
<thead>
<tr>
<th>Virus</th>
<th>Total (n)</th>
<th>%</th>
<th>M/F</th>
<th>Age (Days) Median +/- SD</th>
<th>Clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>3</td>
<td>23</td>
<td>2/1</td>
<td>55 2673</td>
<td>2 bronchiolitis cases*; one neonate who had never left the hospital</td>
</tr>
<tr>
<td>Norovirus</td>
<td>9*</td>
<td>69</td>
<td>5/4</td>
<td>23 664</td>
<td>Three prematurity, three bronchiolitis* and three cancer cases</td>
</tr>
<tr>
<td>Norovirus + Rotavirus</td>
<td>2</td>
<td>15</td>
<td>2/0</td>
<td>70 49</td>
<td>One prematurity case; one neonate who had never left the hospital</td>
</tr>
<tr>
<td>Norovirus +Astrovirus</td>
<td>1</td>
<td>8</td>
<td>1/0</td>
<td>8</td>
<td>Prematurity case</td>
</tr>
</tbody>
</table>

- Bronchiolitis cases correspond to neonates who were hospitalized without any other specific underlying diseases and who developed classical signs of gastroenteritis more than 96 hours after the time of hospitalization

- M= male

- F=female

(- ): not available
Number of Norovirus positive cases in children aged 0-24 months appeared to be significantly higher than that observed in children aged 25-28 months (* $P=0.022$); no other significant variation of frequency was observed for the other viral parameters between the age groups.

**Figure 2:** Distribution of the number of children positive for enteric viruses detection in stool samples following age groups.
Legend of Figures and tables

Figure 1. Distribution (A) and seasonality (B) of Rotavirus, Norovirus, Astrovirus and Adenovirus Positive Antigenic Detection by EIA or ELISA Assays among 973 Stool Samples of Hospitalized Children in Northern France

A- Distribution (%) of the total number of single (n=335) and mixed (n=32) viral intestinal infections among 973 Stool Samples of Hospitalized Children in Northern France. Using the combination of rapid EIA assays and ELISA assays we identified 34% (335/973) of single infections and 3.2% (32/973) of multiple infections; resulting in 37.2% of positive viral detection in stools of infants with gastroenteritis signs during a one year prospective study. Only one stool sample was tested for each patient. (Noro= norovirus; Rota = rotavirus; Adeno=adenovirus; Astro=astrovirus).

B- Monthly distribution of the number of rotavirus, adenovirus, astrovirus, norovirus and multiple infections among the tested children. The total number (n) of tested children per months is indicated at the bottom of the graph.

Figure 2. Distribution of the number of children positive for enteric viruses detection in stool samples following age groups:
Number of Norovirus positive cases in children aged 0-24 months appeared to be significantly higher than that observed in children aged 25-28 months (*P=0.022); no other significant variation of frequency was observed for the other viral parameters between the age groups.

Table 1. Virological, demographic and clinical characteristics of the 15 cases of nosocomial infections identified among the 367 documented viral gastrointestinal infections

M= male
F=female
(- ): not available