Detection of sporadic cases of *Norovirus* infection in hospitalized children in Italy

Sonia Caracciolo, Chiara Minini, Domenico Colombrita, Ida Foresti, Manuela Avolio, Giorgio Tosti, Simona Fiorentini, Arnaldo Caruso

Department of Applied and Experimental Medicine, Section of Microbiology, University of Brescia Medical School, Brescia, Italy

This study was performed to investigate the role of Noroviruses (NV) in sporadic cases of acute diarrhoea among hospitalized children in Brescia Hospital. NV were the most frequently involved viruses (23.7%) and were more common in children >5 years (23/63) than in children <5 years (6/59). The majority of the NV-positive specimens belonged to genotype II (GII). The frequency of rotavirus, enteric adenovirus and astrovirus was 12.2%, 1.6% and 2.4%, respectively. Results obtained confirm the relevance of NV as a causative agent of pediatric diarrhoea and highlight the need for continued surveillance of NV to prevent and control virus spreading.

KEY WORDS: Gastroenteritis, Norovirus, Molecular epidemiology

Gastroenteritis remains a major public health issue worldwide, especially among children (Giordano *et al.*., 2001). More than 700 million cases of acute gastroenteritis are estimated to occur every year in children under the age of 5 years with a mortality of 3.5-5.0 million per year (Snyder and Merson, 1982).

Many different pathogens have been found in the stools of children with gastroenteritis. From them, bacteria, such as *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. among others, and viruses, such as rotaviruses, adenoviruses (Diamanti *et al.*, 1996) and astroviruses (Donelli *et al.*, 1993) have been clearly established as etiologic agents of gastroenteritis in children. More recently, Noroviruses (NV formerly known as “Norwalk-like viruses”) have also been included as a common cause of outbreaks and sporadic cases of gastroenteritis worldwide in individuals of all ages. NV are a group of single-stranded positive-sense RNA viruses belonging to the Caliciviridae family, which includes a large number of genetically related strains. NV can be divided into three distinct genogroups: GI, GII, and GIII (Ando *et al.*, 1995).

GI and GII noroviruses infect humans and include 5 and 10 genetic clusters, respectively; GIII noroviruses infect pigs and cows. Thanks to the development of sensitive molecular assays (e.g., reverse transcription-polymerase chain reaction), and enzyme-linked immunosorbent assays (ELISA), NV have recently been reported as a major cause of viral gastroenteritis outbreaks in different countries (Lopman *et al.*, 2002; Liu *et al.*, 2006).

NV are highly infectious agents whose transmission remains primary faecal-oral, though airborne and fomite transmission may also contribute to the virus spreading during outbreaks. It is often difficult to link secondary or tertiary cases resulting from person-to-person contact to a specific mode of transmission (Fretz *et al.*, 2005). Nevertheless very few studies so far have

Corresponding author
Arnaldo Caruso
University of Brescia Medical School
Piazzale Spedali Civili, 1
25123 Brescia, Italy
E-mail: caruso@med.unibs.it
evaluated the contribution of NV to sporadic pediatric gastroenteritis, as compared to other more common gastroenteritis-associated viruses and bacteria (Boga et al., 2004). The aim of this study was to evaluate the relative contribution of NV to sporadic cases of pediatric gastroenteritis over an extended time period in children seeking medical care for acute diarrhoea in Brescia Children’s Hospital. We also investigated the frequency of norovirus genotypes I and II detection and the presence of viral co-infections.

Between October 2003 and August 2005, a total of 139 children under 14 years old with the clinical symptoms of acute diarrhoea were referred to Brescia Children’s Hospital. Diarrhoea was defined as the occurrence of three or more unformed (loose or watery) stools over a 24h period. Stool specimens were collected during the acute phase of infection. All samples were obtained by direct deposition in a sterile container, then taken to our laboratory within 2h and inoculated into medium on the day of collection for bacteriological culture. Specimens for virus were aliquoted and stored at -80°C until tested. The study included only fecal specimens from sporadic cases.

In order to assess the distribution of infection in pediatric patients, the children were grouped into two categories: those under 5 years of age (48.9%) and those aged 5-14 years (51.1%). Conventional bacterial culture procedures were carried out to isolate gastroenteritis-associated bacteria. Commercial immunoassays IDEIA™ Astrovirus, and IDEIA™ Norovirus (Dako Ltd., Glostrup, Denmark) were used according to the manufacturer’s instructions to detect specific antigens from astrovirus and group I and II noroviruses, respectively. Latex agglutination assays PASTOREX® Rotavirus (Bio-Rad, Hercules, USA) and Diarlex® Adenovirus (Orion Diagnostica, Espoo, Finland) were used according to the manufacturer’s instructions to detect specific antigens from group A rotaviruses and adenoviruses type 40 and 41, respectively.

Among the 139 stool specimens from children with gastroenteritis, 67 (48.2%) contained at least one identifiable infectious agent. Individual enteropathogenic bacteria were isolated from 17 specimens (12.2%). Among the bacteria, Salmonella enterica serovar Enteritidis (6.4%) and Salmonella enterica serovar Typhimurium (1.4%) were those isolated most frequently (7.9%), followed by Campylobacter jejuni (4.3%). No pathogenic parasite was detected in the tested specimens. All specimens that tested negative for culturable bacterial pathogens (n = 122) were then studied for the presence of viruses. Enteropathogenic viruses were detected in 50 out of 122 specimens (40.9%) and, among these, NV were the most frequent (23.7%). Interestingly, both GI and GII virus types were detected, but genotype II (GII) prevailed over genotype I (GI) (86.2% vs 13.8%, respectively).

The other enteric viruses found were rotaviruses (12.2%), astroviruses (2.4%) and adenoviruses (1.6%) (Figure 1). Only 2 specimens (1.6%)
showed a dual viral infection with the concomitant detection of astroviruses and NV. It is interesting to note that 66.6% of all astrovirus-positive specimens (2 out of 3) were found in co-infection with NV. The age of the individuals was then analyzed. In children under the age of 5 years viruses and bacteria were detected with a different frequency (27.9% versus 13.2%, respectively), a finding which was even more striking in children aged 5-14 years (42.2% versus 11.2%, respectively).

The increased frequency of the virus in children from the second group was due exclusively to NV (Table 1). In fact, NV were the most frequently detected viruses in children aged 5-14 years (74% of all norovirus-associated cases), followed by rotaviruses (33.3% of all rotavirus-associated cases). On the other hand, rotaviruses were the most frequently detected viruses in children under the age of 5 (66.6% of all rotavirus-associated cases) followed by NV (26% of all norovirus-associated cases). Adenoviruses were detected only in specimens of children under 5 years old.

This study clearly indicates that NV are the most frequent viral agents in hospitalized children suffering from acute diarrhoea (40.2%) and confirms the role of such viruses in gastroenteritis, as highlighted by several other studies (Widdowson et al., 2005). Because of the high genetic diversity of NV, molecular characterization of genotypes in the strains causing pediatric gastroenteritis is essential to account for norovirus epidemiology. Antigen detection enzyme-linked immunosorbent assays, which are based on the use of monoclonal antibodies to numerous recombinant GI and GII noroviruses capsid proteins, are now available for that purpose.

Characterization of the norovirus genotypes detected in our study showed the presence of both types, though genotype II (GII) prevailed over genotype I (GI) (86.2% vs. 13.8%). The reasons for the high prevalence of the former genotype are unknown, but identification of the viral determinants conductive to its adaptation and virulence may help to explain why the GII genotype has become globally prevalent. Human astroviruses have been increasingly identified as worldwide agents of infantile gastroenteritis (Glass et al., 1996; Matsui and Greenberg, 2001).

Being associated with focal outbreaks of diarrhoea, their incidence in children with gastroenteritis is usually 2-9% (Matsui and Greenberg, 2001). A pivotal molecular epidemiological study of astrovirus infection among Italian children with gastroenteritis showed on overall incidence of 3.1% (De Grazia et al., 2004). Our findings confirm the low astrovirus incidence in children with acute diarrhoea but they also highlight the frequent detection of NV in astrovirus-associated cases. With the exception of one specimen (in which astroviruses were the only detected virus) the other two astrovirus-positive specimens also showed a concomitant presence of GI or GII noroviruses. Further studies are needed to establish whether the association of astroviruses and NV is a common feature in patients with acute gastroenteritis.

The high detection rate of NV causing paediatric diarrhoea reported in this study suggests that they should be included in routine screenings of sporadic cases of acute gastroenteritis. Timely and accurate testing for NV would provide physicians with valuable data and prevent the unnecessary use of antibiotics, with the added cost of prolonged hospitalization. NV are highly infectious agents whose transmission remains primary faecal-oral, though airborne and fomite transmission may also contribute to the virus spreading during outbreaks. It is often difficult to link secondary or tertiary cases resulting from person-to-person contact to a specific mode of transmission. Prompt identification of infected individuals is also beneficial for the surveillance of NV and the adoption of suitable instruments for preventing and controlling virus spread.

### TABLE 1 - Frequency of adenovirus, rotavirus, norovirus and astrovirus in 122 children with diarrhoea by age group in Brescia Children’s Hospital. October 2003-August 2005.

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<thead>
<tr>
<th>Viral Agents</th>
<th>Age range (years)</th>
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<tbody>
<tr>
<td></td>
<td>&lt;5 (n=59)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>2</td>
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<tr>
<td>Rotavirus</td>
<td>9</td>
</tr>
<tr>
<td>Norovirus</td>
<td>6</td>
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