ORIGINAL ARTICLE

Epidemiological aspects of human rotavirus infection in children hospitalized with acute gastroenteritis in an area of northern Italy

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Abstract. Human rotavirus (HRV) is recognized as the most common cause of severe gastroenteritis in children under 5 years of age. Due to the lack of recent reports about the surveillance of HRV infection in Italy, in this study we assessed the prevalence rate of HRV infection on 1,340 stool samples belonging to 1,264 pediatric patients hospitalized with acute gastroenteritis in the period January 2000-December 2002. The stool samples were submitted to virological investigations by electron microscopy (EM) and conventional cell culture, as well as from January 2002 by RT-PCR for norovirus detection. Reovirus-like particles observed by EM were identified by electropherotyping. Single HRV infections were detected in 302 cases (23.9%, ranging from 19.1% in 2000 to 30.2% in 2001). Mixed infections were observed in 28 cases in which HRV was found to be associated with adenovirus in 16 cases (1.3%), with picornavirus in 4 (0.3%), and with norovirus in 8 (2.1% of the 388 cases examined in 2002). The 3 major epidemic periods of HRV infections were March-May 2000 (66 cases), December 2000-May 2001 (128 cases) and September 2001-April 2002 (105 cases) with peaks in March, January and March, and January, respectively. In the periods of major incidence, single HRV infection accounted even for 52.5% of the gastroenteritis cases monthly examined. According to age distribution, 68.9% (208 cases) of HRV infected children was under 4 years (69.6%: 230/330 cases, including mixed infections) and 16.9% (51 cases) was in the 5-12-year age group. The epidemiological aspects of HRV infection, also compared to other enteric virus infections, will contribute to assess the magnitude of the problem of HRV in different settings and to devise strategies for intervention.

Key words: Epidemiology, human rotavirus, acute gastroenteritis, hospitalized children

Introduction

Viral intestinal infections are the most common cause of acute infectious diarrhea in the pediatric population. Worldwide estimates indicate a mean between 7 and 30 episodes of diarrhea during the first 5 years of life, and over 11,000 deaths per day throughout the world (1, 2). In the developed countries, acute diarrhea is a major cause of morbidity in childhood, and substantial medical and healthcare costs are associated with the illness (3).

Human rotavirus (HRV) is the most prevalent agent responsible for acute diarrhea in young children worldwide. Other viral agents such as calicivirus (no-

rovirus and sapovirus), enteric adenovirus, astrovirus and, more recently, torovirus have also been identified thanks to the development of new rapid molecular methods of viral diagnosis (4, 5).

The virion of HRV contains an 11-segment double-stranded RNA genome within a triple-layered capsid. Upon polyacrylamide gel electrophoresis, these 11 segments of dsRNA display a migration pattern called electropherotype which is believed to be characteristic of each individual strain. This methodology has been widely used for epidemiological studies of HRV.

To date, the epidemiology of HRV infection in hospitalized children in Italy has rarely been assessed.

The aim of this study was to survey enteric viral infections, with particular regard to HRV in comparison with other viral agents.

Materials and methods

Patients and samples

A total of 1,340 stool samples was collected from 1,264 hospitalized pediatric patients (median age 2 years 5 months) admitted with acute gastroenteritis to the Major Hospital of Parma, Northern Italy, from January 2000 to December 2002. All specimens were prepared in 10% suspension in phosphate-buffered saline (PBS, pH 7.2), clarified by low-speed centrifugation and submitted to electron microscopy (EM) and conventional cell culture (CCC) for the detection of viral agents. All the 418 stool specimens belonging to 388 patients referring to January-December 2002 were also examined by nested RT-PCR (nRT-PCR) for the detection of norovirus RNA.

Electron microscopy

EM was performed essentially as previously described using standard techniques. Briefly, the suspension was concentrated with a polyacrylamide absorbent gel (Sigma, Italy) and a drop of the suspension was incubated for 25 s on a Formvar carbon-coated grid and then stained with 2% phosphotungstic acid (pH 6.5) for 25 s. The grids were viewed on a Philip's EM208S electron microscope at an initial screen magnification of X 45,000.

Electropherotyping

For electropherotyping separation, reovirus-like positive fecal suspensions were ultracentrifuged for 1 hr at 25,000 rpm by using a Beckman SW41 rotor at 4°C (Beckman Coulter, USA). The dsRNA viral genome was extracted from the concentrated fecal suspensions by using a standard phenol-clorophorm extraction method, followed by ethanol precipitation. The electropherotyping separation of genomic RNA was performed on a 10% polyacrylamide running gel with a 4% stacking gel, followed by silver staining as described by Herring et al. (6).

Conventional cell culture

Aliquots of the stool suspensions were inoculated into cell monolayers for conventional virus isolation. Identification of cytopathogenic agent was performed by EM and/or neutralization assay with Lim and Benyesch-Melnick antisera pools.

Nested RT-PCR for norovirus detection

RNA was rapidly extracted from 250 μl of stool suspension by "EXTRAgen Kit" (Amplimedical, Bioline Division group, Italy) according to the manufacturer's protocol. RT was carried out using random primer "RT-Kit random primers" (Amplimedical), starting from 10 μl of extracted RNA. The first round of nPCR was performed with the primer pair JV12/JV13 (7) in a Progene thermal-cycler (Techne, UK) with the following protocol: 94°C for 2 min, 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min. The second round was done with nested primers designed and evaluated by us in a previous study (data not shown) and performed in analogous cycling conditions with an annealing temperature of 55°C. Amplification products were electrophoresed in 4% ethidium bromide agarose gel and visualized on a UV transilluminator. The selected inner primers yielded a product of 176 bp.

Results

Study of prevalence

Out of the 1,264 children with gastroenteritis, single HRV infections were identified in 302 cases (23.9%): 81 cases in 2000 (19.1% of 423 cases), 137 cases in 2001 (30.2% of 453 cases) and 84 cases in 2002 (21.6% of 388 cases) (Table 1). Overall, in the three-year surveillance there were also 98 cases (7.7%) with adenovirus infection, 29 cases (2.3%) with picornavirus infection and 5 cases (0.4%) with reovirus infection. Moreover, norovirus was identified in 46 cases, all but one from January to December 2002 when specific nRT-PCR was used, revealing an annual prevalence of norovirus infection of 11.6% (45 out of 388 cases).

Mixed infections were observed in 37 cases. HRV was found to be associated with adenovirus in 16 cases (1.3%), with picornavirus in 4 (0.3%), and with norovirus in 8 (2.1% of the 388 cases examined in 2002). The other mixed infections were adenovirus-norovirus in 1.5% (6 out of 388 cases) and picornavirus-norovirus in 0.8% (3 out of 388 cases).

Electropherotyping analysis of the 330 HRV strains detected showed the typical 4-2-3-2 gene segment pattern of group-A HRV: 26 (7.9%) had a short pattern and the remaining 304 (92.1%) a long one (Figure 1).

Seasonality

The major epidemic periods of HRV infection were in March-May 2000 (66 cases), December 2000-May 2001 (128 cases) and September 2001-April 2002 (105 cases) (Figure 2). Apparently, HRV infection was absent in January, June and October 2000, as well as in July 2001 and 2002. For each major epidemic period, the peak was reached in March 2000, January and March 2001, and January 2002, respectively. In the same epidemic periods, within the gastroenteritis cases monthly examined, single HRV infections accounted for up to 52.1% (37 cases) in March 2000, 52.5% (21 cases) in April 2001 and 47.5% (29 cases) in January 2002. Electropherotypes with short patterns were found from March to August 2000 (7 short vs 63 long patterns) and from April to June 2001 (19 short vs 15 long patterns), with a peak in April (14 cases) when they became the dominant pattern.

Looking at the other viral infections, adenovirus infection was more homogeneously distributed throu-

Table 1. Prevalence of viral infections in pediatric patients hospitalized with gastroenteritis from January 2000 to December 2002

	Year						Total	
	2000		2001		2002			
N° of samples	447		475		418		1340	
N° of patients	423		453		388		1264	
N° of patients positive for:								
rotavirus	81	19.1%	137	30.2%	84	21.6%	302	23.9%
adenovirus	40	9.5%	36	7.9%	22	5.7%	98	7.8%
picornavirus	6	1.4%	14	3.1%	9	2.3%	29	2.3%
reovirus	2	0.5%	2	0.4%	1	0.3%	5	0.4%
norovirus*	-	-	1^	0.2%	45°	11.6%	46	n.d.
rotavirus + adenovirus	6	1.4%	7	1.5%	3	0.8%	16	1.3%
rotavirus + picornavirus	3	0.7%	0	0.0%	1	0.3%	4	0.3%
rotavirus + norovirus*	-	-	-	-	8	2.1%	8	n.d.
adenovirus + norovirus*	-	-	-	-	6	1.5%	6	n.d.
picornavirus + norovirus*	-	-	-	-	3	0.8%	3	n.d.

* Norovirus was detected by nRT-PCR starting from January 2002

^ Calicivirus-like particles detected by EM were identified as norovirus by nRT-PCR

° Calicivirus-like particles were detected by EM in 4 cases

n.d.: not determined because pertinent to a one-year study

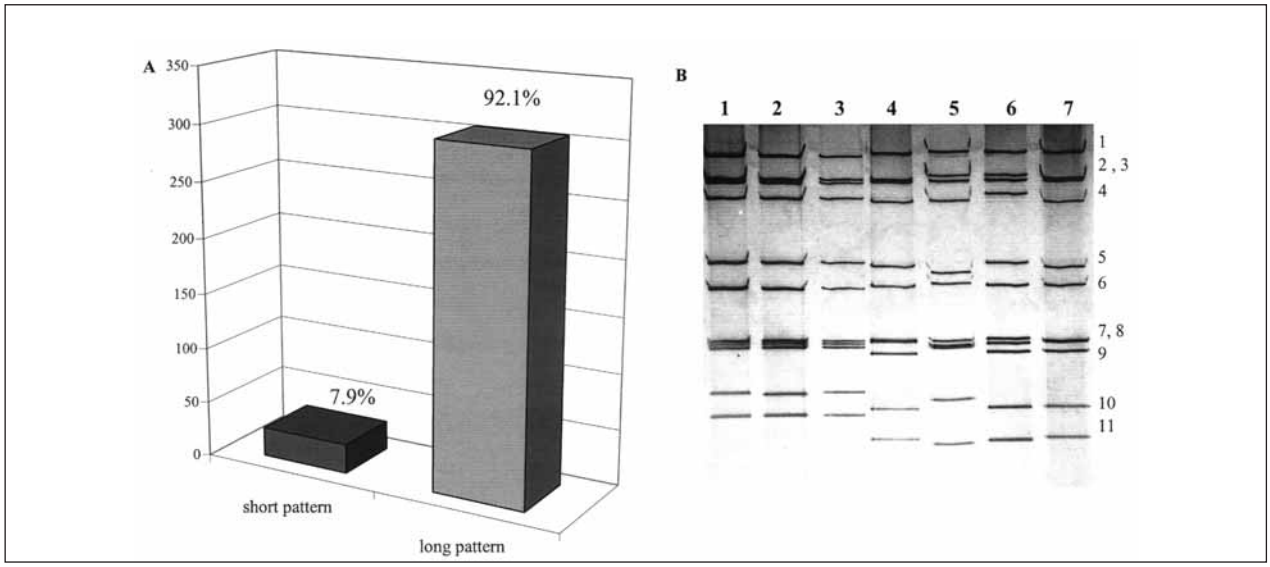

Figure 1. Genomic electropherotypes of group-A rotavirus strains in a three-year surveillance (January 2000-December 2002). A) Distribution of 330 rotavirus strains by RNA segment pattern. B) Representative genomic electropherotypes. Lanes 1, 2 and 3 show short pattern rotaviruses; lanes 4, 5, 6 and 7 show long pattern rotaviruses. Numbers on the right indicate the 11 segments of dsRNA.

ghout the year, with a major epidemic period from October 2000 to June 2001, peaking in November 2000 (11 cases) and June 2001 (7 cases). Picornavirus infection was less frequent in 2000 (9 cases) than in 2001 (14 cases) and 2002 (13 cases), and reovirus was rarely detected in 2000-2002. When considering 2002, norovirus infection showed its major epidemic period from September to December (43 cases), peaking in November (16 cases).

Age distribution

Out of the 302 HRV positive patients (median age 21 months), 68.9% (208 patients) were < 4 years old, 16.9% (51 patients) were 5 to 12 years old, and the age of the remaining 14.2% (43 patients) was unknown (Figure 3).

Out of the 16 cases with HRV-adenovirus mixed infection, 11 cases (68.75%) were in the 4 year age group, 3 cases (18.75%) in the 5-12 year age group, and 2 cases (12.5%) in the unknown age group. The 4 patients with HRV-picornavirus mixed infection were < 4 years old in 3 cases and 5 years old in 1 case. All 8 HRV-norovirus mixed infections were shown in children aged < 4 years.

Discussion

During a three-year (January 2000-December 2002) surveillance of enteric infections in hospitalized pediatric patients, we found a prevalence rate of single HRV infections of 23.9% (ranging from 19.1% in 2000 to 30.2% in 2002), in comparison with 11.6% of single norovirus (referring only to the 2002 surveillance) and 7.7% of single adenovirus infection (ranging from 5.7% in 2002 to 9.5% in 2000). These data, according to the worldwide prevalence range for hospitalized children, confirm the importance of HRV as the main viral pathogen of enteric disease in pediatric patients, apparently followed by norovirus, as reported in other industrialized countries (8). Overall, single infections due to other viral agents (picornavirus and reovirus) accounted for a minor prevalence rate (2.7%).

HRV gastroenteritis severe enough to require hospitalization occurs more frequently in infants and young children (9). This tendency was clearly observed in this study, since the highest incidence of HRV gastroenteritis (68.8% at least) was found in the under 4 year old group. Interestingly, norovirus gastroenteritis had its highest incidence (69.5%) in the same age

group. Apparently, in this age group HRV and norovirus infections were more frequent than adenovirus infection (63.3%).

Dual infections are generally not expected and often misdiagnosed. Therefore, data about concomitant viral infections vary widely in literature. In our

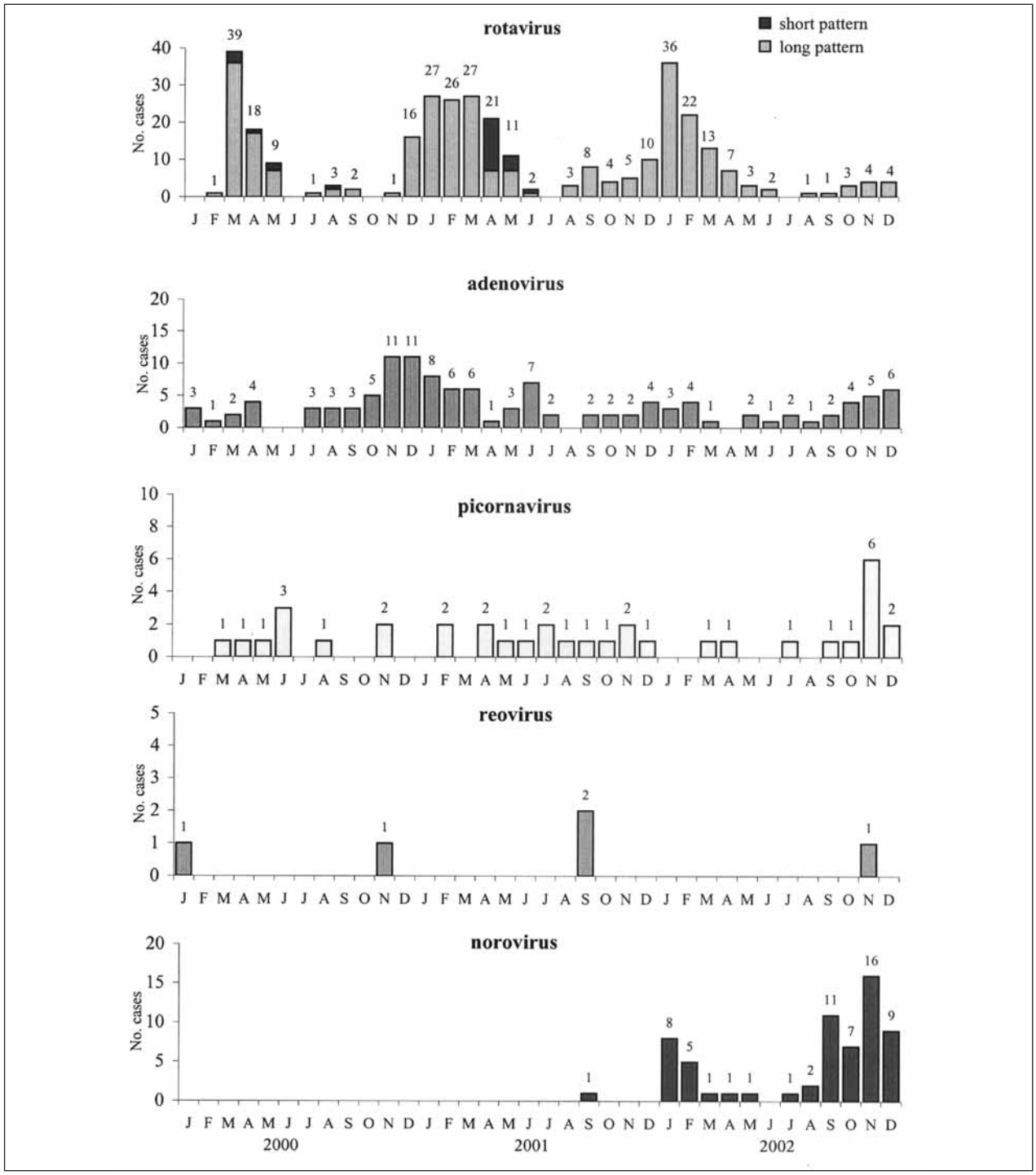

Figure 2. Distribution by month of rotavirus in relation to other gastroenteritis viruses from January 2000 to December 2002.

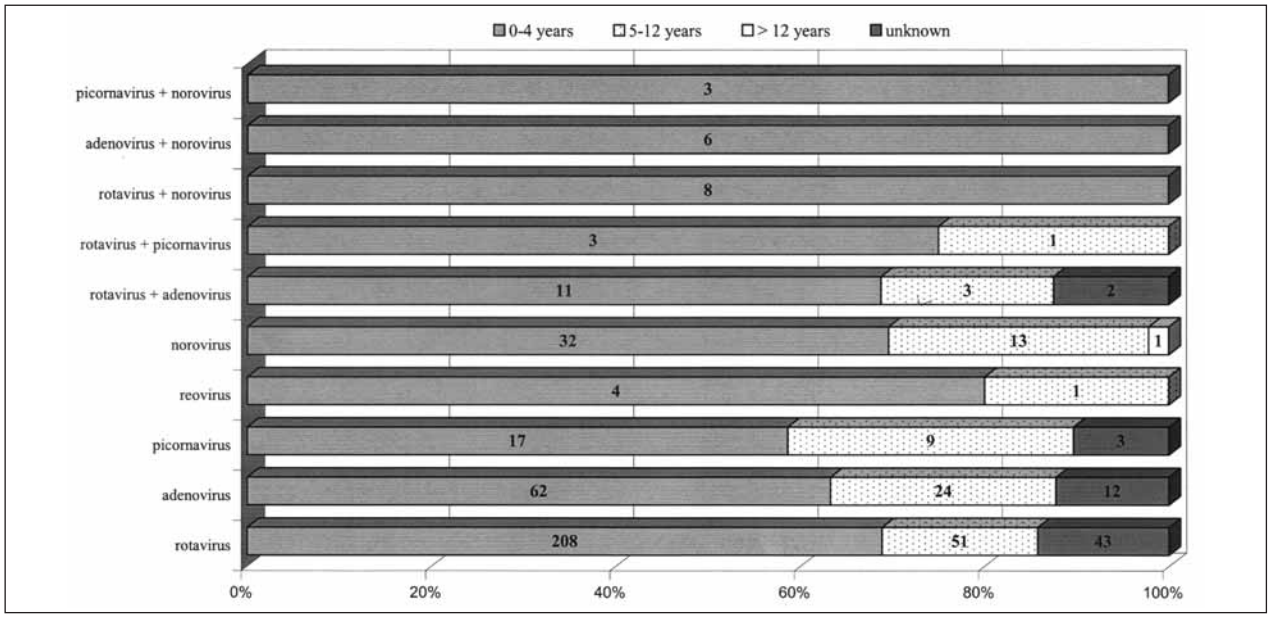

Figure 3. Distribution of viral infections according to pediatric patients hospitalized with gastroenteritis from January 2000 to December 2002.

analyses, mixed infections were detected in 2.9% (37 cases), and HRV was the most frequently implicated viral agent (28 cases: 75.7%). The most frequent viral co-infection was HRV-norovirus (8 cases: 21.6%), observed in children under 4 years of age.

As described in other temperate climate countries, HRV infection presented a seasonal pattern, with a major incidence in winter (43.0%: 142 cases) and spring (44.8%: 148 cases), and with a few cases also during the summer months. Compared to the seasonal patterns of adenovirus and norovirus, the HRV season seems to start and peak later.

As expected, all 330 HRV strains analyzed by electrophoresis showed the characteristic 4-2-3-2 pattern of group-A HRV. Most of them (92.1%) had a long profile, with the exception of 26 strains (7.9%), for which a short profile was visualized in two consecutive years, with a peak in April 2001, when HRV with short pattern became dominant (66.7%: 14/21). The short profile has been demonstrated to be characteristic of almost all antigenic-subgroup-I HRV, which are almost always serotype-G2 strains (9, 10).

Our epidemiological data, along with information on antigenic and molecular details of HRV strains, will contribute to assess the magnitude of the problem of HRV in different settings and to set priorities for intervention, such as vaccine-based prevention strategies.

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