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ORIGINAL ARTICLE

Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents

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Abstract A 22-month study (2008-2009) was carried out on 273 patients (average age 40 months), admitted with gastroenteritis to the Pediatric Unit of L. Sacco University Hospital in Milan, Italy. Fecal samples were investigated for rotavirus (HRV), norovirus (NoV), adenovirus (AdV), sapovirus (SaV), enterovirus, astrovirus and bocavirus (HBoV). A 38.3% incidence of infection was observed for HRV, followed by NoV (16.2%), HBoV (13.6%), AdV (2.6%) and SaV (0.6%). Clinical evaluation of 109 gastroenteritis patients with confirmed diagnosis was graded by the Ruska-Vesikari scoring system, showing vomiting (78%), diarrhea (96%) and fever (80%). A total of 25 NoV-positive samples were selected for nucleotide sequence analysis. The severity of AdV-associated infection was lower than for NoV, HRV and HBoV. These latter viruses caused similar symptoms that were indistinguishable using clinical information. NoV, HRV and HBoV were often present as mixed infections (13.1%). Sequencing of NoV-positive samples allowed identification of GII.2, GII.3 and GII.4 2006 variants.

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Introduction

Gastroenteritis agents are associated with a high morbidity, especially in infants and young children, and cause large outbreaks and large numbers of sporadic cases worldwide. Currently, a national surveillance system that is able to determine the incidence of different etiologic agents of gastroenteritis is not in place in Italy. However, reporting systems based on regional data have been implemented in the last few years [1, 2]. In the past, human rotavirus (HRV; family *Reoviridae,* genus *Rotavirus*) was considered to be responsible for most acute diarrheas in children [3, 4]. More recently, improved diagnostic tools have shown that norovirus (NoV; family *Caliciviridae*, genus *Norovirus*) also has a major role in both epidemic and sporadic cases of gastroenteritis and is considered to be the primary cause of viral gastroenteritis in all age groups throughout the world. Sapovirus (SaV; family *Caliciviridae*, genus *Sapovirus*) causes sporadic cases and outbreaks of gastroenteritis, mainly in young children <5 years of age [5]. More recently, human bocavirus (HBoV; family *Parvoviridae*, genus *Bocavirus*) has been discovered, and it has been suggested to be involved in a large spectrum of clinical manifestations, including gastroenteritis [6].

The impact of these new enteric viruses on the epidemiology of infantile gastroenteritis in the northern Italian region of Lombardy is scarcely known due to the lack of a systematic approach to testing patients' clinical samples and analyzing diagnostic data [3, 7]. Estimates of the incidence of enteric infections normally suffer from large

underreporting in Italy; in fact, the last comprehensive overall data on acute gastroenterical infections in Italy were obtained by Ruggeri and Declich in 1999 [8]. In addition, there is also poor information regarding the clinical and etiological role of each individual virus in cases of co-infection.

In this study, the epidemiological and clinical role of HRV, NoV, adenovirus (AdV; family *Adenoviridae*, genus *Mastadenovirus*), enterovirus (family *Picornaviridae*, genus *Enterovirus*), astrovirus (family *Astroviridae*, genus *Mamastrovirus*), SaV and HBoV were evaluated in sporadic pediatric gastroenteritis patients attending the pediatric unit of L. Sacco University Hospital in Milan. Molecular characterization of circulating NoV strains was also performed.

Material and methods

Population study and clinical evaluation

From January 2008 to October 2009, 273 children, suffering from acute gastroenteritis, in the age range from 0 to 222 months (samples average age 40 months, males 57%) were examined. These children mainly lived in the northwesten areas of Milan, which is the most populated city in Lombardy and the traditional area served by L. Sacco University Hospital.

Stool (407) specimens obtained from 273 patients were collected and sent to the clinical microbiology laboratory of the L. Sacco University Hospital. Demographic (age and sex) and clinical data (days of hospitalisation, vomiting, diarrhoea, fever and percentage of dehydration) that were useful for our investigation were gathered for only 154 out of 273 children (Fig. 1).

A dataset form was filled out for signs and symptoms, adapted from the clinical severity scores for HRV gastroenteritis according to the Ruska-Vesikari (RV) scoring system [7, 9], including the daily presence of fever, vomiting, and daily frequency and duration of diarrhea. Clinical information was obtained from the virology request forms and from case sheets. The severity of gastroenteric disease [9, 10] was determined using a numerical score for clinical severity of diarrhoeal episodes, as reported: minor (1-8), moderate (9-14) and serious (>15).

Stools were processed to isolate *Salmonella, Shigella, Campylobacter, Yersinia*, and *Clostridium difficile*. All samples were also routinely processed for HRV by enzymatic immunoassay card testing (Meridian, Cincinnati, OH, USA). Samples were stored at −80°C until used, and they were then analyzed for NoV, HBoV, AdV, astrovirus, SaV and enterovirus.

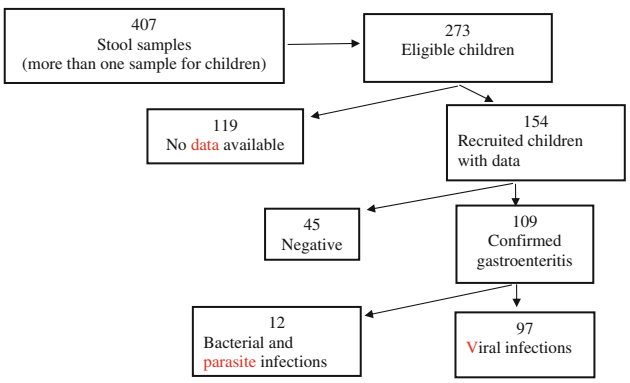

Fig. 1 Flowchart of the children enrolled in the study

Immunoassay detection

The commercial rapid *in vitro* qualitative ImmunoCard-STAT! Rotavirus test (Meridian) was used for detection of HRV. Patient stools were diluted 1:15 in the kit sample diluent, and 150 µl of the suspension was added to the device sample port. The signal generated by mobilization of monoclonal-antibody-coated gold particles through the polyclonal anti-HRV antibody (test) zone was considered a positive HRV detection result in the presence of a negative control reading.

Virological investigation

Viral RNA (NoV, enterovirus) and DNA (AdV and HBoV) were extracted from 200 µl of stool sample using the commercial kits QIAamp® Viral RNA Blood Mini Kit and QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany), respectively. RNA and DNA were eluted in 60 µl of AE Buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 9). NoV ("Norovirus TaqMan real time RT-PCR kit"; Argene, Verniolle, France), AdV (AdV r-gene; Argene), HBoV (HBoV r-gene; Argene) and enterovirus (enterovirus r-gene; Argene) were processed by commercial real-time kits. The NoV test was able to detect theGI and GII genogroups. Amplification was performed using an ABI 7300 instrument using the amplification temperature conditions indicated by the manufacturer (Applied Biosystems, Warrington, UK). Negative samples were tested further using a Multiplex PCR that allows simultaneous amplification of target DNA/cDNA of human enteric AdV, group A HRV, astrovirus and NoV GI/GII (Seeplex Diarrhoea ACE Detection, Seegene, Seoul, Korea).

Samples that were found to be negative by the previous method were investigated for SaV. RNAs were subjected to reverse transcription and PCR amplification (RT-PCR) using a Superscript RT-PCR one-step kit (Invitrogen, Carlsbad, CA, USA), and primers p289-p290, which anneal to a 330-nt conserved region of the RNA-dependent polymerase gene (ORF1) [11]. The samples that were found to be

negative with this test were further analyzed using primers SV5317-SV5749, which anneal to a 433-nt region of the human SaV capsid protein primer (ORF1) [12].

Sequencing of NoV

Stools that were found to be positive for NoV by real-time PCR were also tested by conventional RT-PCR using two primer pairs, G1SKF-G1SKR and G2SKF-G2SKR, which amplify a 338-bp fragment of the viral genome ORF2 of genogroup I and II NoV, respectively [13]. The ten DNA fragments that were obtained were purified by silica membrane using a QIA Quick Gel Extraction Kit (QIAGEN), and subjected to sequence analysis using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction Kit, version 3.1 (PE Applied Biosystems). The sequences obtained were compared with those of the NCBI and FBVE (FoodBorne Viruses in Europe [https://hypocrates.rivm.nl/Divine-Event/index.html]) data banks, using DNASIS Max software (Hitachi Software Engineering Company, Alameda, CA, USA).

Statistical analysis

Two different statistical approaches were undertaken to test possible associations between patients' age and frequency of infection, and possible differences between the clinical conditions of patients infected with different viral agents. SaV was not included in this analysis because there was only a single record in the database. A chi-square test was performed on a contingency table to analyze viral agents (NoV, AdV, HBoV, HRV and enterovirus) versus age class (age interval 36 months). A MANOVA analysis was also performed imposing the different viruses as a factor and using the following response variables: hospitalization length (days), duration of vomiting (days), maximum number of vomiting episodes per day, duration of diarrhea (days), maximum number of diarrhea episodes per day, fever intensity, percentage of dehydration, dehydration score and RV scoring system. The analysis was performed excluding the variables that did not show homogeneity of variance (Levene test) even after a square root or logarithmic transformation. Moreover, a linear discriminant analysis (LDA) was subsequently performed in order to identify patterns of distinction between the different viruses. The analyses were performed using the software SPSS Statistics 17.0.

Results

Etiological evaluation

One hundred fifty-four out of 273 children, aged on average about 40 months, examined in the clinical microbiology laboratory of the L. Sacco University Hospital were enrolled in the study: in 109/154 (70.8%), the clinical diagnosis of infectious gastroenteritis was confirmed by laboratory findings. Of the 109 patients affected by gastroenteritis associated with pathogens, 9 cases were due to bacteria (5.8%), and 3 cases to parasites (1.9%). Viruses were detected in 99/154 patients (64.3%). In particular, 86/154 viral infections were mono-infections (55.8%) and 13/154 cases were mixed infections (8.4%). Overall, HRV was the pathogen most frequently detected (38.3% of 154 investigated patients), followed by NoV (16.2%), HBoV (13.6%) AdV (2.6%) and SaV (0.6%). Enterovirus and astrovirus were not detected. In the mono-infections, the positive detection rates of different enteric viruses for the total number of patients were 31.2% for HRV, 12.3% for NoV, 9.1% for HBoV, 2.6% for adenoviruses and 0.6% for SaV. Regarding dual infection cases, NoV/*C. difficile* and *Campylobacter*/HRV were detected in 2 cases, HRV/HBoV were present in 6 patients (3.9%), HRV/NoV were present in 4 patients (2.6%), and HBoV/NoV in 1 patient.

Clinical signs and duration of symptoms

One hundred nine patients with a diagnosed pathogen supporting gastroenteritis were graded by the RV scoring system. Minor symptoms were scored in 24 (22%) patients, moderate in 50 (46%) and serious in 35 (32%) out of the 109 total evaluated cases (Table 1). For 119 children, pediatricians were not able to obtain further clinical information from the parents of these patients.

Vomiting was reported in 85 patients (78 %), and diarrhea was reported in 87 patients (87%); the average length of vomiting and diarrhea was 2.4 days and 4.2 days, respectively, with a frequency of 4 and 6 events per days, respectively. As a general rule, duration of symptoms decreased with age, independent of the virus detected. The only exceptions were the mean duration of vomiting and diarrhoea for HBoV. Stool formation showed variation: 5 children had bloody feces, 38 children had liquid feces, 41 children had semi-liquid stools and 3 children had mucous feces. Body temperature was normal in 20 patients (18.3%), 18 patients (16.5%) had a 1-point increase; 22 (20.2%) had a 2-point increase and 44 (40.4%) had a temperature of more than 38.9°C. In 5 patients, fever was not recorded. Concerning dehydration, the patients had the following scores: 0 in 41 cases (37.6%), 1 in 13 cases (11.9%) and 2 in 49 cases (45.0%). In 6 patients, dehydration was not evaluated. The overall average score for dehydration was 1.1.

Clinical characteristics of children infected with NoV, HRV, AdV and HBoV are shown in Table 1. Diarrhea was present in all mono-infection cases, and vomiting was present in all 19 patients who were positive for NoV, in 45

Table 1 Symptoms and duration of illness with viral agents according to age, for hospitalized patients with mono- and mixed-infection gastroenteritis in the L. Sacco University Hospital

Virus	Total cases	Age group (months)		Hospital. days	Vomit dur. (days)	Max. no. vomit/day	Diarrhoea dur. (days)	Diarrhoea max./days	Fever (°C)	% dehydr.	RV
Monoinfections											
HRV	48	1–18	Mean	6	2	4	4	7	2.2	2.9	14.0
			s.d.	2	2	4	2	3	0.9	2.8	2.9
		19–36	Mean	5	3	7	4	6	2.2	1.5	13.0
			s.d.	2	5	6	2	5	1	2.0	3.4
		>37	Mean	5	2	4	4	5	1.1	0.5	10.3
			s.d.	1	2	6	2	3	1	1.2	3.6
Nov	19	1–18	Mean	6	3	4	5	5	1.7	3.1	12.9
			s.d.	3	2	4	2	2	1.4	3.5	3.5
		19–36	Mean	4	2	3	3	7.	1.7	1.6	12.7
			s.d.	2	1	3	1	2	1.2	2.1	2.1
		>37	Mean	4	1	2	2	3	0.3	1.4	8.0
			s.d.	1	1	3	2	2	0.6	2.4	3.5
HbV	14	1–18	Mean	2	2	4	6	2	2.8	11.5	10.3
			s.d.	2	2	3	4	1	3.8	4.5	3.6
		19–36	Mean	4	2	5	6	6	3	5.3	15.5
			s.d.	1	2	7.	1	1	0	0.1	3.5
		>37	Mean	6	5	2	7	4	1.6	3.3	12.0
			s.d.	2	6	2	5	3	1.3	4.0	4.6
AdV	4	1–18	Mean	10	0	0	4	5	2.5	0.2	7.5
			s.d.	5	0	0	1	1	0.7	0.1	2.1
		>37	Mean	5	0	0	1	6	3	0	9.0
			s.d.	–	–	–	–	–	–	–	–
Mixed infections											
HbV/HRV	6	1–18	Mean	4	2	3	4	8	2.0	0.7	13.0
			s.d.	1	1	3	2	5	1.2	1.0	3.7
		19–36	Mean	3	3	10	5	5	3.0	5.3	18.0
			s.d.	–	–	–	–	–	–	–	–
NoV/HRV	4	1–18	Mean	10	3	4	4	5	3	3.0	15.0
			s.d.	–	–	–	–	–	–	–	–
		19–36	Mean	6	1	2	4	6	3	0.0	12.0
			s.d.	–	–	–	–	–	–	–	–
		>37	Mean	4	1	1	2	2	0.5	0.0	7.0
			s.d.	0	1	1	3	3	0.7	0.0	4.2
NoV/HbV	1	1–18	Mean	9	6	4	10	6	3	12.6	19.0
			s.d.	–	–	–	–	–	–	–	–

The distribution of viral agents was based on 86 cases with monoinfections and on 11 with mixed infections. A single case of SaV was detected but not reported. Bacterial infections are not reported. Where standard deviation is not reported, a single case per category was counted

of 48 (93.7%) of the patients positive for HRV and in 9 of 14 (64%) patients positive for HBoV. No vomiting was present in AdV infection cases. Fever was reported in 15 of 19 (78.9%) and 8 of 14 (57%) patients infected with NoV and HBoV, respectively, and in 42 of 48 (87.5%) HRV cases. The percentages for dehydration were similar for all of the viruses detected. The average RV scoring system was 12.3 in all mono-infection gastroenteritis cases. The dehydration percentage in cases of mixed infections (Table 1) was overall more severe, particularly in NoV-HBoV- and HRV-HBoV-affected patients. Four of 14 (30.8%) gastroenteritis patients infected with HBoV showed respiratory difficulties.

MANOVA analysis indicated the presence of a significant difference between the clinical patterns associated with different viruses (Wilk's Lambda = 0.50, df = 27,

$p < 0.001$). On this basis, a discriminant analysis (DA) was performed considering all of the variables. The clinical pattern shown by AdV was substantially different and was discriminated by the analysis (100% correct reclassifications), while HRV (54% correct reclassifications), NoV (47% correct reclassifications) and HBoV (63% correct reclassifications) were undistinguishable (data not shown). The variables that mostly discriminated the virus strain were the RV scoring system, maximum number of diarrhea episodes per day, diarrhoea duration and fever intensity. Moreover, a variable that specifically distinguished AdV from the other different viruses was the absence of vomiting episodes. However, the discriminating influence of this variable was negligible for the other viruses, and its weight in the overall model was less than that of the other variables.

Statistical analysis of virus infection rates among the different age classes was performed on 99 patients. The average age of occurrence of NoV was about 25 months (median value 11 months), 32 months (median value 21 months) for HRV, 41 months (median value 14 months) for HBoV and 19 months (median value 8 months) for AdV (Table 2).

A chi-square test performed for each virus (except AdV, due to the low frequency of occurrence) confirmed a higher peak of infection in the age class 0-36 months for HBoV (Pearson's contingency coefficient = 16.90, df = 18, *p*-value <0.0001), NoV (Pearson's contingency coefficient = 40.82, df = 3, *p*-value <0.0001), HRV (Pearson's contingency coefficient = 64.48, df = 5, *p*-value <0.0001). The small number of mixed infections did not allow any statistical evaluation of their association with age classes.

Figure 2 shows the monthly distribution of sporadic gastroenteritis cases in 2008 and 2009. Sporadic HRV infections were observed between January and May 2008 (24 cases) with a peak in January-February (18 cases), and in 2009, a first peak was observed in February-April (24 cases). A second HRV peak was observed in June 2009 (4 cases). Most of the NoV-positive cases occurred from September to December 2008, with a peak of positivity detected in October 2008 (7 cases) and March 2009 (4 cases). HBoV cases were observed between November 2008 and February 2009. AdV was detected in January (1 case), April (1 case), September (1 case), and December (1 case). SaV was detected in October 2009 (1 case).

Molecular sequencing analysis

Sequence analysis on ten NoV strains revealed that all belonged to GII. Six viruses were closely related to the GII.4 2006 variant 2006b, and two were related to the GII.4 2006 variant 2006a, which were reported to circulate broadly since the second and the first semester of 2006, respectively. Both virus GII.4 2006a and 2006b were detected in this study in both 2008 and 2009. GII.2 and GII.3 NoV strains were detected in two patients in June and July 2009, respectively. The GII.4 variant 2006b was detected only in males, while the genotypes GII.4 variant 2006a was equally distributed in both sexes. For all genotypes, the RV scoring system showed a moderate mean value around 13,3.

Discussion

Acute gastroenteritis is a leading cause of childhood morbidity and mortality in developing countries [14]. While HRV had been established for a long time as the major cause of severe gastroenteritis in infants and young children worldwide, NoV has more recently gained increasing recognition as an important cause of severe gastroenteritis in childhood [15] and has been detected in children admitted to hospital at rates varying between 3% and 30%, both in developed and developing countries [2, 16, 17]. Nevertheless, it should be noted that a potential underestimation of the prevalence of HRV may occur due to the different sensitivities of commonly used tests [18]. However, even taking into account this potential underestimation, the HRV prevalence estimated in this study is similar to those of similar trials and investigations in Italy [3, 19].

Table 2 Absolute frequency of infection with different viruses in different age groups

Age group (months)	Virus						
	AdV	HBoV	NoV	HRV	Any virus	Negative	Total
0–36	3	16	21	43	83	23	106
37–72	1	2	1	9	13	10	23
73–108	0	1	0	5	6	5	11
109–144	0	0	2	0	2	2	4
145–180	0	0	1	2	3	3	6
181–216	0	1	0	0	1	0	1
217–252	0	1	0	0	1	2	3
Total	4	21	25	59	109	45	154

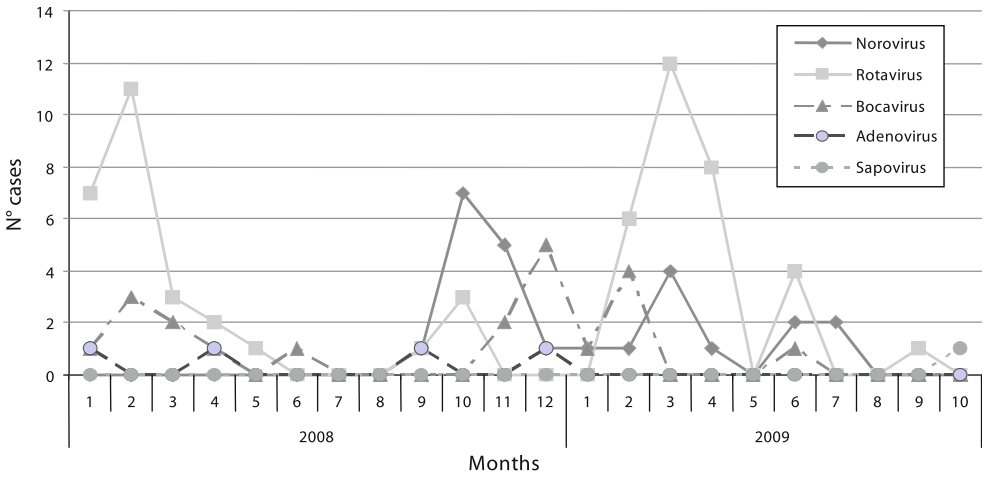

Fig. 2 Monthly distribution of sporadic cases of gastroenteritis during 2008-2009

Such variation could be due to different hygienic conditions in different countries, to different surveillance systems and to different methods used in diagnostic laboratories [16, 17]. In a recent Italian study, NoV emerged as the main cause of viral enteritis, being responsible for 39% of diarrhea cases reported for children admitted to the hospital in Palermo [20].

The epidemiology of viral gastroenteritis in Italy is largely unknown, due to the absence of a nationwide surveillance system for diarrheal diseases. Thus, this study was designed to assess the prevalence of viral agents among pediatric cases in order to improve knowledge in the social and geographical context of Lombardy. This study confirms previous surveys indicating that HRV is the major cause of severe pediatric acute gastroenteritis in Italy [3, 8, 19], being found in approximately 38.3% of patients investigated, alone or in combination with other agents. Other viruses were detected in approximately 32.3% of patients, and of these, NoV was the most common (16.2%), followed by HBoV (13.6%) and AdV 2.6%. SaV was found in one case. Altogether, these agents accounted for 64.3% of all patients admitted with acute gastroenteritis. No enterovirus or astrovirus was detected. NoV, HRV and HboV were often present in mixed infections (13.1%).

Concerning the clinical manifestations associated with the different infectious agents, patients with gastroenteritis caused by HRV, NoV, HBoV and AdV showed, in general, a moderate score of severity. Fever and dehydration percentages were the most discriminating variables, in particular for AdV, which showed the lowest mean values, and this indicates a clinical pattern that can possibly be detected without the use of virological testing.

Despite efforts to adopt a broad-spectrum virological diagnostic approach, microbial agents have not been identified in a large percentage of gastroenteritis cases, even if clinical data were not available from all of the children enrolled. NoV and HRV were the viruses most frequently detected in cases of mixed infections, also in combination with HBoV. These results are consistent with other investigations on the viral etiology of pediatric gastroenteritis [21–24]. Overall, patients with mixed infection presented more severe clinical features, and this is possibly due to a synergistic action of multiple pathogens, both in terms of clinical severity and duration of symptoms. In particular, a severe disease score was obtained with HBoV in mixed infection with NoV and HRV. A high incidence of mixed HBoV infection with either respiratory or gastrointestinal viruses in children with or without respiratory disease has been reported in different studies, but the clinical significance is still not clear [25]. The same authors showed that HBoV infection alone seems to be associated with mild symptoms in children with acute gastroenteric disease, and that infection becomes severe only when it is associated with other viral pathogens. Furthermore, Jie-Mei and co-workers [26] reported that co-infection with HBoV did not increase the clinical symptoms of HRV gastroenteritis. A preferential infection of children belonging to specific age groups has been reported for some gastroenteritis viruses [2, 3], and the same trend was observed in the present study for the younger age groups. Moreover, this trend appears to be common to all of the viruses detected, although for all of the viruses, a somewhat higher prevalence was observed in younger age groups.

The GII.4 NoV variant 2006b was the most frequent NoV strain detected, accounting for 6 of 10 noroviruses sequenced. The other five patients were infected with three distinct NoV genotypes (GII.4 2006a, GII.2 and GII.3). The co-circulation of three different genotypes of NoV is consistent with the genetic diversity reported in other studies [3]. No NoV genotype was associated with particular clinical symptoms or severity scores.

Some differences in the seasonality of different viral agents were observed during the two years of this investigation. HRV incidence had a peak in either February or March, confirming previous studies [1]. NoV incidence was highest in October 2008 and in March 2009; HBoV showed a peak of incidence in November-December 2008 and was not detected in the same period of 2009. In general, viral gastroenteritis appears to occur mostly during the coldest seasons.

Investigation of other viruses such as parechoviruses, torovirus, aichivirus, picobirnavirus and picotrinavirus [28–30] might help to increase the rate of diagnosis of children with gastroenteritis.

References

1. Colomba C, Saporito L, Giammanco GM, De Grazia S, Ramirez S, Arista S, Titone L (2007) Norovirus and gastroenteritis in hospitalized children, Italy. Emerg Infect Dis 9:1389–1391
2. Medici MC, Martinelli M, Abelli LA, Ruggeri FM, Di Bartolo I, Arcangeletti MC, Pinardi F, De Conto F, Izzi G, Bernasconi S, Chezzi C, Dettori G (2006) Molecular epidemiology of norovirus infections in sporadic cases of viral gastroenteritis among children in northern Italy. J Med Virol 78:1486–1492
3. Medici MC, Martinelli M, Arcangeletti MC, Pinardi F, De Conto F, Dodi I, Virdis R, Abelli LA, Aloisi A, Zerbini L, Valcavi P, Calderaro A, Bernasconi S, Izzi GC, Dettori G, Chezzi C (2004) Epidemiological aspects of human rotavirus infection in children hospitalized with acute gastroenteritis in an area of northern of Italy. Acta Biomed 75:100–106
4. Iritani N, Seto Y, Kubo H, Murakami T, Haruki K, Ayata M, Ogura H (2003) Prevalence of Norwalk like virus infection in cases of viral gastroenteritis among children in Osaka City, Japan. J Clin Microbiol 41:1756–1759
5. Hansman G, Saito H, Shibata C, Oseto M, Oka T, Taeda N (2007) Outbreaks of gastroenteritis due to sapovirus. JCM 45:1347–1349
6. Allander T (2008) Human bocavirus. J Clin Virol 41:29–33
7. De Grazia S, Giammanco GM, Colomba C, Cascio A, Arista S (2004) Molecular epidemiology of astrovirus infection in Italian children with gastroenteritis. Clin Microbiol Infect 10:1006–1035
8. Ruggeri FM, Declich S (1999) Rotavirus infection among children with diarrhoea in Italy. Acta Pediatr Suppl 88(426):66–71
9. Ruska T, Versikari T (1990) Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis 22:259–267
10. Rockx B, de Wit M, Vennema H, Vinjé J, de Bruin E, van Duynhoven Y, Koopmans M (2002) Natural history of human calicivirus infection: a prospective cohort study. Clin Inf Dis 35:246–253
11. Farka T, Zhong WM, Jing Y, Huang PW, Espinosa SM, Martinez N, Morrow AL, Ruiz-Palacios GM, Pickering LK, Jiang X (2004) Genetic diversity among sapovirus. Arch Virol 149:1309–1323
12. Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamrin P, Tonusin S, Okitsu S, Nishio O, Takeda N, Ushijima H (2004) Genetic diversity of Norovirus and Sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. JCM 42(3):1305–1307
13. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K (2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100:107–114
14. Lopman BA, Reacher MH, Vipond IB, Hill D, Perry C, Halladay T, Brown DW, Edmunds WJ, Sarangi J (2004) Epidemiology and cost of nosocomial gastroenteritis Avon England 2002–2003. Emerg Infect Dis 10:1827–1834
15. Moreno-Espinosa S, Farkas T, Jiang X (2004) Human caliciviruses and pediatric gastroenteritis. Semin Pediatric Infect Dis 15:237–245
16. McIver CJ, Hansman G, White P, Doultree JC, Catton M, Rawlinson WD (2001) Diagnosis of enteric pathogens in children with gastroenteritis. Pathology 33:353–358
17. Bereciartu A, Bok K, Gómez J (2002) Identification of viral agents causing gastroenteritis among children in Buenos Aires, Argentina. J Clin Microbiol 25:197–200
18. Dennehy PH, Heartin M, Nelson SM, Reising SF (1999) Evaluation of the ImmunoCardSTAT! Rotavirus assay for detection of group A rotavirus in fecal specimens. J Clin Microbiol 37:1977–1979
19. De Grazia S, Ramirez S, Giammanco GM, Colomba C, Martella V, Lo Biundo C, Mazzola R, Arista S (2007) Diversity of human rotaviruses detected in Sicily, Italy, over a 5-year period (2001–2005). Arch Virol 152:833–837
20. Colomba C, Saporito L, Giammanco GM, De Grazia S, Ramirez S, Arista S, Titone L (2007) Norovirus and gastroenteritis in hospitalized children, Italy. Emerg Infect Dis 9:1389–1391
21. Iritani N, Seto Y, Kubo H, Murakami T, Haruki K, Ayata M, Ogura H (2003) Prevalence of Norwalk like virus infection in cases of viral gastroenteritis among children in Osaka City, Japan. J Clin Microbiol 41:1756–1759
22. Chiba S, Sakuma Y, Kogasaka R, Akihara M, Horino K, Nakao T, Fukui S (1979) An outbreak of gastroenteritis associated with calicivirus in an infant home. J Med Virol 4:249–254
23. Cunliffe NA, Booth JA, Elliot C, Lowe SJ, Sopwith W, Kitchin N, Nakagomi O, Nakagomi T, Hart CA, Regan M (2010) Healthcare associated viral gastroenteritis among children in a large pediatric hospital, United Kingdom. Emerg Infect Dis 16(1): 55–62
24. Simpson R, Aliyu S, Iturriza-Gómara M, Desselberger U, Gray J (2003) Infantile viral gastroenteritis: on the way to closing the diagnostic gap. J Med Virol 70(2):258–256
25. Esposito S, Bosis S, Niesters HG, Tremolati E, Sabatini C, Porta A, Fossali E, Osterhaus AD, Principi N (2008) Impact of human bocavirus on children and their families. J Clin Microbiol 46:1337–1342
26. Yu JM, Li DD, Xu ZQ, Cheng WX, Zhang Q, Li HY, Cui SX, Miao-Jin Yang SH, Fang ZY, Duan ZJ (2008) Human bocavirus infection in children hospitalized with acute gastroenteritis in China. J Clin Virol 42(3):280–285
27. Lindell AT, Grillner L, Svensson L, Wirgart BZ (2005) Molecular epidemiology of norovirus infection in Stockholm, Sweden, during the year 2000 to 2003: association of the GGIIb genetic cluster with infection in children. J Clin Microbiol 43:1086–1092
28. Greninger AL, Runchel C, Chiu CY, Haggerty T, Parsonnet J, Ganem D, De Risi JL (2009) The complete genome of Klassevirus—a novel picornavirus in pediatric stool. Virol J 6:1–9
29. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff R (2009) A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog 5(4):e1000391
30. Pham NT, Trin QD, Khamrin P, Maneekarnn N, Shimizu H, Okitsu S, Mizuguchi M, Ushijima H (2009) Diversity of human parechoviruses isolated in stool samples collected from Thai children with acute gastroenteritis. J Clin Microbiol 48(1): 115–119