Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Diagnostic Microbiology and Infectious Disease 77 (2013) 231–235

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Virology

Molecular detection of gastrointestinal viral infections in hospitalized patients

Francesca Rovida, Giulia Campanini, Antonio Piralla, Kodjo Messan Guy Adzasehoun, Antonella Sarasini, Fausto Baldanti *

S.S. Virologia Molecolare, S.C. Virologia e Microbiologia, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy

ARTICLE INFO

Article history:
Received 15 March 2013
Received in revised form 10 July 2013
Accepted 31 July 2013
Available online 12 September 2013

Keywords:
Gastrointestinal viruses
Real-time RT-PCR
Gastroenteritis
Stool samples

ABSTRACT

Gastrointestinal viral syndromes are a common cause of morbidity and mortality in humans worldwide. Etiological agents include a large number of viruses encompassing several orders, families, and genera. During the period April 2011 to April 2012, 689 stool samples from as many patients hospitalized at the Fondazione IRCCS Policlinico San Matteo of Pavia exhibiting gastrointestinal syndromes were examined for the presence of rotavirus, norovirus, astrovirus, adenovirus, rhinovirus, enterovirus, parechovirus, bocavirus, coronavirus, sapovirus, cosavirus, and aichi virus using polymerase chain reaction assays. Gastrointestinal viral agents were detected in 246 (36%) patients of the 689 analyzed. Adenovirus and norovirus were the most common viruses in this cohort, while aichi virus was the only gastrointestinal agent not detected. Surprisingly, rhinovirus was one of the most frequently detected viruses. However, a potential association with gastroenteritis remains to be confirmed.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Viral gastroenteritis is a common cause of morbidity and mortality in humans worldwide, affecting all age groups. Etiological agents include a number of viruses encompassing several orders, families, and genera.

Viral pathogens causing acute gastroenteritis include *Rotavirus* (RV), members of the *Caliciviridae* family such as *Norovirus* (NoV) and *Sapovirus* (SaV), *Adenovirus* (HAdV) and *Astrovirus* (HAstV) (Eckardt and Baumgart, 2011). Viral gastroenteritis can be more severe in young children, the elderly, and immunocompromised patients. RV causes 600,000–875,000 deaths per year (Clark and McKendrick, 2004), the majority of which occur in developing countries, and it is the most severe and common cause of diarrhea in children under 5 years of age (Wilhelmi et al., 2003). Frequently, NoVs are responsible for outbreaks and sporadic cases of nonbacterial gastroenteritis in children and adults worldwide (Kele et al., 2011). SaV is considered an important cause of gastroenteritis in children under 5 years of age, while it is of minor importance in adults (Eckardt and Baumgart, 2011). HAstVs and enteric HAdVs cause gastroenteritis, primarily in children less than 4 years of age (Dennehy, 2011).

Human bocavirus and human coronavirus, mainly involved in infections of the respiratory tract, are also implicated in gastrointestinal infections (Clark and McKendrick, 2004; Khan and Bass, 2010).

Members of the *Picornaviridae* family, for example, enterovirus (EV), parechovirus (HPeV), aichi virus (AiV), and human cosavirus (HCoSV), are causative agents of gastroenteritis (Harvala et al., 2010; Holtz et al., 2008). Recently, also rhinoviruses (HRV) have been detected in stool samples (Harvala et al., 2012).

Considering the highly contagious nature of these viruses, surveillance of new cases is needed for outbreak prevention and control. Unfortunately, the number of agents implicated in gastrointestinal infections makes the construction of a comprehensive diagnostic panel very challenging. The present study is aimed at evaluating the circulation of gastrointestinal viruses in hospitalized patients using polymerase chain reaction (PCR) assays.

2. Methods

2.1. PCR assays

Overall, 689 stool samples stored in the period April 2011 to April 2012 from as many patients (356 pediatrics and 333 adults) with gastrointestinal syndromes hospitalized at the Fondazione IRCCS Policlinico San Matteo of Pavia (a teaching and university hospital with 50,000 admissions, 2,500,000 outpatients visits, and 94,000 emergency consultations per year) were systematically examined for the presence of gastroenteric viruses. Gastrointestinal syndrome was defined as the rapid onset of 2 or more of the following symptoms: diarrhea, vomiting, nausea, fever, or abdominal pain. In the present study, stool samples collected from patients with diarrhea during the acute phase of gastroenteritis were retrospectively analyzed.

In more detail, all 689 samples were tested by: i) real-time reverse transcriptase polymerase chain reaction (RT-PCR) for NoV, RV, HAstV, EV, HRV, HPeV, SaV, human coronavirus (hCoV); ii) real-time PCR for HAdV; iii) nested RT-PCR for AiV and HCoSV; and iv) nested PCR for human bocavirus (hBoV).

* Corresponding author. Tel.: +39-0382-502420; fax: +39-0382-502599.
E-mail address: f.baldanti@smatteo.pv.it (F. Baldanti).

0732-8893/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.diagmicrobio.2013.07.020

Table 1
Molecular parameters used for detection of different gastrointestinal viruses in stool samples.

Virus	molecular test	Gene target	Thermal profile	Cycle no.	Oligonucleotide sequence (5′→3′)	References
NoV GI	Real-time RT-PCR	capsid	50 °C/10′ 95 °C/10′ 95 °C/15″ 60°/1′	1 45	QNIF4: CGCTGGATGCGNTTCCAT NV1LCR: CTTAGACGCCATCATCATTTAC NV1LCpr probe: TGGACAGGAGAYCGCRATCT	Da Silva et al. (2007)
NoV GII	Real-time RT-PCR	ORF1-ORF2 junction	50 °C/10′ 95 °C/10′ 95 °C/15″ 60°/1′	1 45	QNIF2d: ATGTTCAGRTGGATGAGRTTCTCWGA COG2R: TCGACGCCATCTTCATTCACA QNIFS probe: AGCACGTGGGAGGGGATCG	Da Silva et al. (2007)
RV	Real-time RT-PCR	non-structural protein 3	50 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 50	ROTAs (fwd1): ACCATCTTCACGTAACCCTC ROTAs (fwd2): ACCATCTACACATGACCCTC ROTAas (rev): CACATAACGCCCCTATAGCC ROTA (probe): ATGAGCACAATAGTTAAAAGCTAACACTGTCAA	Van Maarseveen et al. (2010)
HAstV	Real-time RT-PCR	ORF-1a	50 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 50	ASTVs: TCTYATAGACCGYATTATTGG ASTVas: TCAAATTCTACATCATCACCAA ASTV probe: CCCCADCCATCATCATCTTCATCA	Van Maarseveen et al. (2010)
EV	Real-time RT-PCR	5′-noncoding region	45 °C/10′ 95 °C/10′ 95 °C/15″ 60°/1′	1 40	rhientfwd: CCTCCGGCCCCTGA P1.4taq: GATTGTCACCATAAGCAGCC Entpr1 probe: CGGAACCGACTACTTTGGGT	Van Doornum et al. (2007)
HRV	Real-time RT-PCR	5′-noncoding region	45 °C/10′ 95 °C/10′ 95 °C/15″ 60°/1′	1 40	primer fwd: CPXGCCZGCGTGGC primer rev: GAAACACGGACACCCAAAGTA probe: TCCTCCGGCCCCTGAATGYGGC	Lu et al. (2008)
HPeV	Real-time RT-PCR	5′-NTR	50 °C/10′ 95 °C/10′ 95 °C/15″ 58 °C[a]/30″ 72 °C/10″	1 50	AN345: GTAACASWWGCCTCTGGGSCCAAAAG AN344: GGCCCCWGRTCAGATCCAYAGT AN257 probe: CCTRYGGGTACCTYCWGGGCATCCTTC	Nix et al. (2008)
SaV GI GII GIV	Real-time RT-PCR	polyprotein	50 °C/10′ 95 °C/10′ 95 °C/15″ 60°/1′	1 40	sapo.fwdA: ACCAGGCTCTCGCCACCTA sapo.fwdB: ATTTGGCCCTCGCCACCTA sapo.rev: GCCCTCCATYTCAAACACTAWTTT sapo.probeA: CTGTACCACCTATGAACCA sapo.probeB: TTGTACCACCTATGAACCA sapo.probeC: TGTACCACCTATAAACCA sapo.probeD: TGCACCACCTATGAAC	Logan et al. (2007)
hCoV OC43	Real-time RT-PCR	nucleoprotein	45 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 40	OC43 fwd: CGATGAGGCTATTCCGACTAGGT OC43 rev: CCTTCCTGAGCCTTCAATATAGTAACC OC43 probe: TCCGCCTGGCACGGTACTCCCT	Dare et al. (2007)
hCoV 229E	Real-time RT-PCR	nucleoprotein	45 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 40	229E fwd: CAGTCAAATGGGCTGATGCA 229E rev: AAAGGGCTATAAAGAGAATAAGGTATTCT 229E probe: CCCTGACGACCACGTTGTGGTTCA	Dare et al. (2007)
hCoV NL63	Real-time RT-PCR	nucleoprotein	45 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 40	NL63fwd: GACCAAAGCACTGAATAACATTTTCC NL63 rev: ACCTAATAAGCCTCTTTCTCAACCC NL63 probe: AACACGCTTCCAACGAGGTTTCTTCAACTGAG	Dare et al. (2007)
hCoV HKU1	Real-time RT-PCR	replicase 1b	45 °C/10′ 95 °C/10′ 95 °C/15″ 55 °C/1′	1 40	HKU1 fwd: CCTTGCGAATGAATGTGCT HKU1 rev: TTGCATCACCACTGCTAGTACCAC HKU1 probe: TGTGTGGCGGTTGCTATTATGTTAAGCCTG	Dare et al. (2007)
HAdV	Real-time PCR	hexon	50 °C/2′ 95 °C/10′ 95 °C/15″ 60°/1′	1 40	AQ1: GCCACGGTGGGGTTTCTAAACTT AQ2: GCCCCAGTGGTCTTACATGCACATC AP probe: TGCACCAGACCCGGGCTCAGGTACTCCGA	Heim et al. (2003)
AiV	Nested RT-PCR	3CD junction	50 °C/10′ 95 °C/10′ 95 °C/30″ 55 °C/30″ 72°/1′	1 40	6261: ACACTCCCACCTCCCGCCAGTA 6779: GGAAGAGCTGGGTGTCAAGA	Yamashita et al. (2000)
			94 °C/10′ 94 °C/30″ 65 °C/30″ 72 °C/1′ 72 °C/10′	1 35 1	C94b: GACTTCCCCGGAGTCGTCGTCT 246k: GACATCCGGTTGACGTTGAC	Kaikkonen et al. (2010)
HCoSV	Nested RT-PCR	5′NTR	45 °C/20′ 94 °C/5′ 94 °C/1′ 55 °C[b]/1′ 68 °C/45″ 94 °C/1′ 53°/1′ 68°/45″ 68 °C/10′	1 10 40 1	DKV-N5U-F1: CGTGCTTTACACGGTTTTTGA DKV-N5U-R2: GGTACCTTCAGGACATCTTTGG	Kapoor et al. (2008)
hBoV 1-2-3-4	Nested PCR	VP1/2	95 °C/10′ 95 °C/45″ 57 °C[b]/1′ 72 °C/30″ 95°C/30″ 54 °C/30″ 72°C/30″ 72 °C/10′	1 10 30 1	DKV-N5U-F2: ACGGTTTTTGAACCCCACAC DKV-N5U-R3: GTCCTTTCGGACAGGGCTTT	
			95 °C/10′ 95 °C/35″ 58 °C[b]/1′ 72 °C/1′ 95 °C/30″ 54 °C/45″ 72 °C/45″ 72 °C/10′	1 10 30 1	AK-VP-F1: CGCCGTGGCTCCTGCTCT AK-VP-R1: TGTTCGCCATCACAAAAGATGTG	
			95 °C/10′ 95 °C/35″ 60 °C[b]/1′ 72 °C/1′ 95 °C/30″ 58 °C/45″ 72 °C/45″ 72 °C/10′	1 10 30 1	AK-VP-F2: GGCTCCTGCTCTAGGAAATAAAGAG AK-VP-R2: CCTGCTGTTAGGTCGTTGTTGTATGT	Kapoor et al. (2010)

ORF = open reading frame; NTR = nontranslated region; 3CD = C terminus 3C-N terminus 3D junction.
[a] Probe detection during the 58 °C annealing step.
[b] A decrease of 0.5 °C in annealing temperature each cycle.

The age of pediatric patients ranged from 4 days to 16 years (median, 2 years), and the age of adult patients ranged from 17 to 96 years (median, 61 years). This retrospective study was performed according to guidelines of the Institutional Review Board of the Fondazione IRCCS Policlinico San Matteo on the use of biologic specimens for scientific purpose in keeping with Italian law (art.13 D.Lgs 196/2003).

Viral nucleic acids were extracted from 500 μL of 10% fecal suspension, using the automated extractor Nuclisens® easyMAG™ (BioMérieux, Lyon, France) coextracting DNA and RNA in a final elution volume of 55 μL.

PCR methods were performed according to published protocols (Da Silva et al., 2007; Dare et al., 2007; Heim et al., 2003; Kaikkonen et al., 2010; Kapoor et al., 2008; Kapoor et al., 2010; Logan et al., 2007; Lu et al., 2008; Nix et al., 2008; Van Doornum et al., 2007; Van Maarseveen et al., 2010; Yamashita et al., 2000), with slight modifications in the reverse transcriptase protocol as well as in the adoption of 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) for the amplification step. In detail, 5 μL of eluted nucleic acids were submitted to real-time RT-PCR, real-time PCR, nested RT-PCR, or nested PCR assays using the Ag-Path-ID one-step RT-PCR kit (Applied Biosystems) according to manufacturer's indications. The sequence of primers and probes was not modified (Table 1). The PCR thermal profile by Van Maarseveen et al. (2010) and by Heim et al. (2003) were slightly modified (Table 1) without affecting sensitivity and specificity, as internally validated by parallel amplification of positive and negative samples and according the results of the Quality Control for Molecular Diagnostics (www.QCMD.org; Glasgow, Scotland, UK). The performance of molecular assays in comparison with commercial assays has been previously reported (Rovida et al., 2013).

Purified PCR products were sequenced using the BigDye Terminator Cycle-Sequencing kit (Applied Biosystem) with an ABI Prism 3100 DNA sequencer (Applied Biosystem). Sequences were assembled using the Sequencer software, version 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). Nucleotide alignments were constructed using the ClustalW method with MEGA version 4.1 software (Tamura et al., 2007).

3. Results

3.1. Patients' characteristics

Overall, 689 stool samples from as many patients (356 pediatrics and 333 adults) with gastrointestinal syndromes were analyzed. The age of pediatric patients ranged from 4 days to 16 years (median, 2 years), and the age of adult patients ranged from 17 to 96 years (median, 61 years). Gastrointestinal viruses were detected in 246 (35.7%) patients. Among these, a single pathogen was diagnosed in 191 (27.7%) patients, as multiple infections in 55 (8.0%) patients, while in 443 (64.3%) patients, no viruses were detected (Fig. 1A).

3.2. Frequency and distribution of gastrointestinal viruses

The number of patients positive for single or multiple gastrointestinal viruses is reported in Fig. 1B. The most common gastrointestinal virus was HAdV, accounting for 17.5% (43/246) of positive samples. NoV accounted for 16.5% (41/246) of positive samples; RV, for 15.5% (38/246); HRV, for 14.5% (36/246); SaV, for 3.0% (8/246); EV, for 3.0% (7/246); HAstV, for 2.5% (6/246); HCoV, for 2.0% (5/246); HBoV for 2.0% (5/246); HPeV, for 1.0% (2/246); and HCoSV for 0.5% (1/246). None of the patients (0%) tested positive for AiV infection.

Among the HAdV positive samples, serotype 1 was the most frequent (5/43, 11.6%), followed by serotype 2-5-F41 (3/43, 7.0% each). Among calicivirus, NoV was the most frequent at 83.6% (41/49), while SaV was detected in 16.4% (8/49) samples. NoV GII was more frequently diagnosed (40/41, 97.5%) than GI (1/41, 2.5%).

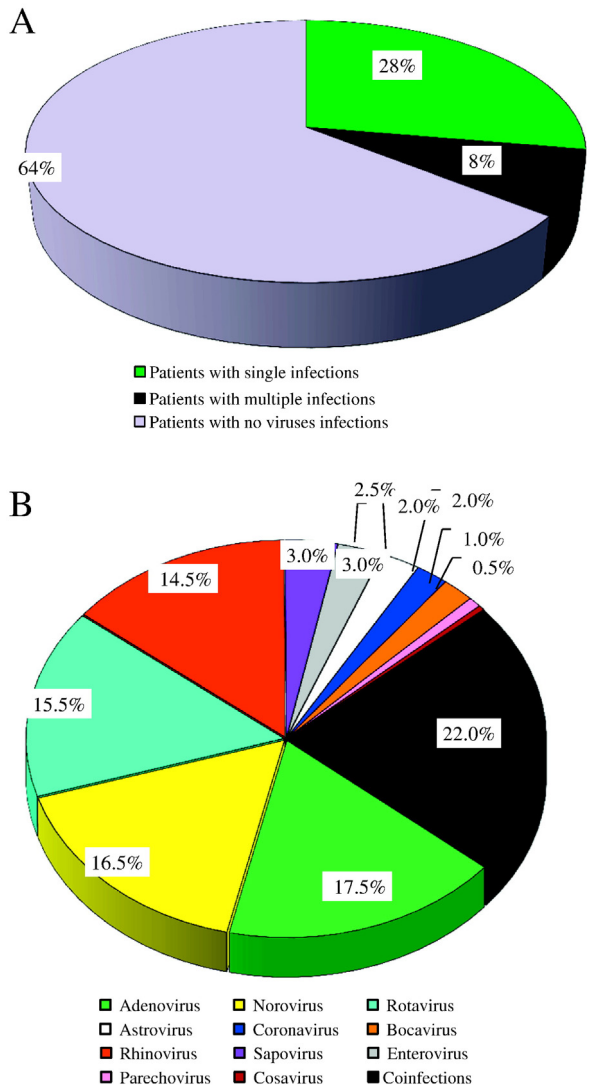

Fig. 1. (A) Number of patients positive for single (n = 191) or multiple (n = 55) gastrointestinal viruses among 689 hospitalized patients with gastrointestinal syndrome. (B) Proportion of patients positive for individual (n = 191) or mixed (n = 55) gastrointestinal virus infections.

Among *Picornavirus*, HRV was the most frequently detected (36/46, 78.3%), EV was detected in 15.2% samples (7/46), HPeV accounted for 4.3% (2/46), and HCoSV corresponded to 2.2% (1/46) of *Picornavirus*-positive samples.

Multiple infections were detected in 22% (55/246) of patient (Fig. 1A and B). Most multiple infections involved HRV (n = 25), HAdV (n = 23), RV (n = 19), NoV (n = 12), and HBoV (n = 12). Multiple infections involved 2–4 viruses at the same time. The most frequently detected coinfections were HAdV + HRV (8/55, 14.5%).

3.3. Distribution of virus gastrointestinal infection in different age groups

The highest number (110/233, 47.2%) of viral gastrointestinal infections was detected in children (<5 years old), while the lowest (44/186, 23.6%) was detected in adults. The gastrointestinal virus distribution in different age groups of patients was 47.2% (110/233) in children aged <5 years, 43.0% (53/123) in children aged 5–15 years, 23.6% (44/186) in patients aged 16–65 years, and 26.5% (39/147) in the elderly >65 years. The most frequent virus infections in children <5 years old were caused by RV (27/110, 24.5%), while coinfections accounted for 33.6% (37/110) of all infections (37/110). NoV infections (20/39, 51.3%) were diagnosed more frequently in adults aged >65

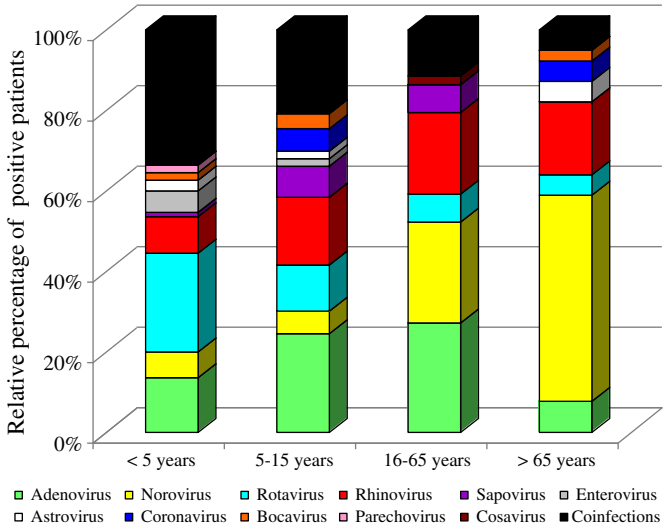

Fig. 2. Distribution of gastrointestinal viruses in hospitalized patients with gastrointestinal syndromes, according to patient age.

years. The relative frequency of gastrointestinal virus positivity in stool samples according to age distribution is listed in Fig. 2.

3.4. Seasonal distribution of gastrointestinal virus infection

During the study period, a cumulative infection peak was reached in the winter season (between January and March) with 48.0% (118/246) of patients testing positive for gastrointestinal viruses (Fig. 3). HAdV was detected throughout the year but was more frequent in October to November and January to February. HRV was present year round, except in September and February, and peaked in May (6/17, 35.3%) and January 13.6% (6/44). RV was detected more frequently from December to March with a peak in February (11/36, 30.5%), while it decreased during the warmest period of the year. NoV was absent in September, November, and December; peaked in March (20/38, 52.6%); and was present during the rest of the year.

4. Discussion

Viral gastroenteritis is one of the most frequent diseases in children and adults and continues to be a significant cause of morbidity and mortality worldwide (Wilhelmi et al., 2003). Diagnostic panels (either molecular or immunologic) should include all virus agents potentially associated with gastrointestinal syndromes but are presently largely incomplete. Detection of gastrointestinal viruses is routinely performed using immunologic assays, such as the enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test (ICT). Nevertheless, these tools have limited sensitivity (Rovida et al, 2013). In recent years, real-time PCR techniques have shown greater sensitivity and specificity than immunological assays for detection of gastrointestinal viruses (Clark and McKendrick, 2004; Rovida et al. 2013; Van Maarseveen et al. 2010; Wilhelmi et al. 2003).

In an attempt to better understand the epidemiology of viral gastroenteritis in hospitalized patients, a 1-year surveillance study was carried out. A limitation of the study is the unavailability of data on other gastrointestinal agents (parasites, bacteria, and fungi). Thus, the results of this study are useful to verify the relative frequency of different viruses in diarrheic stools, while attributing clinical significance to the virologic data would require a larger dataset. Gastrointestinal viruses were detected in about one third of symptomatic patients, a percentage somewhat lower than expected. The distribution of NoV, RV, SaV, HAstV, and HBoV infections is in agreement with data reported in previous surveillance studies in developed countries (González et al., 2011; Medici et al., 2012; Van Maarseveen et al., 2010). However, unlike previous results, HAdV appeared to be the most frequent viral agent. This result could be related to the different sensitivity of assays used. However, monitoring HAdV gastrointestinal diseases in children aged <5 years over a period of 15 years in Japan showed that the annual percentage of HAdV infections ranged from 1.8% to 15.4% indicating yearly fluctuations in HAdV prevalence (Dey et al., 2012). HAdV infection in our study appeared to peak in October suggesting a potential cluster of infection. The investigation of potential local outbreaks is beyond the scope of this study. However, this type of investigation is highly needed and should be implemented.

In the present study, surprisingly, HRV was one of the most frequent viruses in stools of patients with gastrointestinal syndromes.

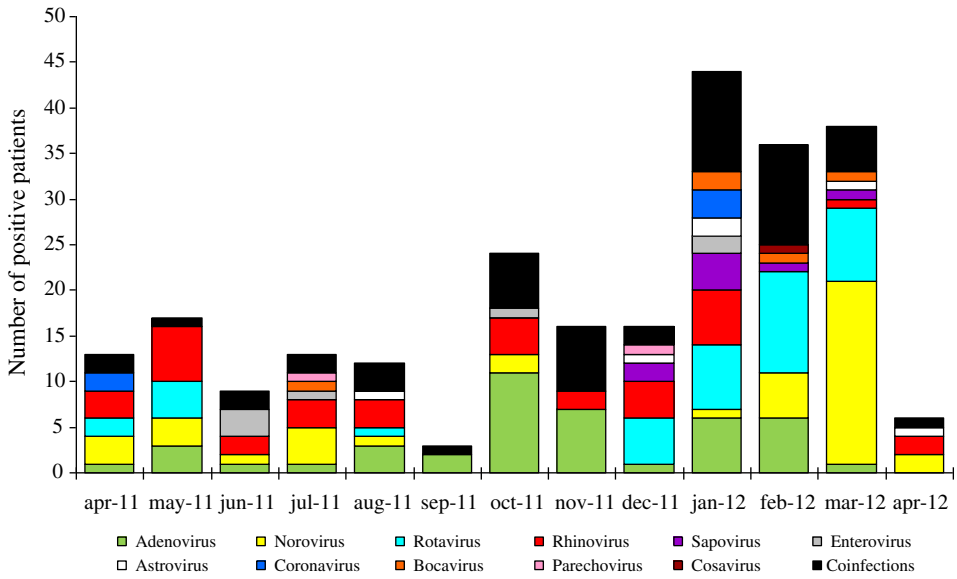

Fig. 3. Frequency and seasonal distribution of gastrointestinal viruses in hospitalized patients with gastrointestinal syndromes.

In addition, HRV was the most frequent virus in multiple infections. Presently, the role of HRV in gastroenteritis is debated (Harvala et al., 2012; Honkanen et al., 2013; Lau et al., 2012). Our data support the presence of HRVs in a significant proportion of patients with gastroenteritis and also in the absence of other gastrointestinal viruses. However, more focused studies, including the systematic detection and culturing of viral, bacterial, and parasitic agents, are needed to understand the role of HRV in stool samples. In this study, several members of the genus *Picornavirus* were detected, including a case of HCoSV gastrointestinal infection, whose clinical characteristics have been reported elsewhere (Campanini et al., 2013). In agreement with published data (González et al. 2011), we found that NoV was the most prevalent of calicivirus, followed by SaV. In addition, NoV GII was more frequently diagnosed than GI.

Multiple infections (involving 2–4 viruses) were detected in about one quarter of patients. These results are in agreement with a previous surveillance study in France (Tran et al., 2010), which reported that, in different European countries, the percentages of mixed viral infection in stool samples ranged from 4.4 to 29%. Furthermore, we observed that multiple infections were detected more frequently in paediatric patients than in adults.

The number of viral gastroenteritis was higher in winter (from January through March) than in other seasons, but HAdV and HRV were detected all year round. NoV infections peaked in March, and RV was detected more frequently during the winter season.

In conclusion, the findings of this study show that HAdV, NoV, and RV are the leading cause of viral gastroenteritis in hospitalized patients. The investigation of all enteric viruses (including HAstV, EV, HPeV, HBoV, HCoV, SaV, HCoSV, and AiV) is needed to improve routine laboratory diagnostics and to provide additional epidemiologic data on the circulation of gastrointestinal viruses in the hospital settings.

Acknowledgments

We thank Daniela Sartori for careful preparation of the manuscript and Laurene Kelly for revision of the English.

This work was supported by the Ministero della Salute, Fondazione IRCCS Policlinico San Matteo, Ricerca Corrente (grant no. 80682). The authors declare no competing financial interest.

References

Campanini G, Rovida F, Meloni F, Cascina A, Ciccocioppo R, Piralla A, et al. Persistent Human Cosavirus infection in lung transplant recipient, Italy. Emerg Infect Dis 2013. (in press).

Clark B, McKendrick M. A review of viral gastroenteritis. Curr Opin Infect Dis 2004;17:461–9.

Da Silva AK, Le Saux JC, Parnaudeau S, Pommepuy M, Elimelech M, Le Guyader FS. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Appl Environ Microbiol 2007;32(4):184–90.

Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. J Infect Dis 2007;196:1321–8.

Dennehy PH. Viral gastroenteritis in children. Pediatr Infect Dis 2011;30:63–4.

Dey SK, Hoq I, Okitsu S, Hayakawa S, Ushijima H. Prevalence seasonality, and peak age of infection of enteric adenoviruses in Japan, 1995–2009. Epidemiol Infect 2012:1–3.

Eckardt AJ, Baumgart DC. Viral gastroenteritis in adults. Recent Pat Antiinfect Drug Discov 2011;6:54–63.

González GG, Liprandi F, Ludert JE. Molecular epidemiology of enteric viruses in children with sporadic gastroenteritis in Valencia, Venezuela. J Med Virol 2011;83:1972–82.

Harvala H, Wolthers KC, Simmonds P. Parechovirus in children: understanding a new infection. Curr Opin Infect Dis 2010;23:224–30.

Harvala H, McIntyre CL, McLeish NJ, Kondracka J, Palmer J, Molyneaux P, et al. High detection frequency and viral loads of human Rhinovirus species A to C in fecal samples; diagnostic and clinical implications. J Med Virol 2012;84:536–42.

Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. J Med Virol 2003;70(2):228–39.

Holtz LR, Finkbeiner SR, Kirkwood CD, Wang D. Identification of a novel picornavirus related to cosaviruses in a child with acute diarrhea. Virol J 2008;5:159–63.

Honkanen H, Oikarinen S, Peltonen P, Simell O, Ilonen J, Veijola R, et al. Human rhinoviruses including group C are common in stool samples of young Finnish children. J Clin Virol 2013;56(3):334–8.

Kaikkonen S, Räsänen S, Rämet M, Vesikari T. Aichi virus infection in children with acute gastroenteritis in Finland. Epidemiol Infect 2010;138:1166–71.

Kapoor A, Victoria J, Simmonds P, Slikas E, Chieochansin T, Naeem A, et al. A highly prevalent and genetically diversified picornaviridae genus in South Asian children. PNAS 2008;105:20482–7.

Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis 2010;201(11):33–1643.

Kele B, Lengyel G, Deak J. Comparison of an ELISA and two reverse transcription polymerase chain reaction methods for norovirus detection. Diagn Microbiol Infect Dis 2011;70:475–8.

Khan MA, Bass DM. Viral infections: new and emerging. Curr Opin Gastroenterol 2010;26:26–30.

Lau SKP, Yip CCY, Lung DC, Lee P, Que T, Lau Y, et al. Detection of human rhinovirus C in fecal samples of children with gastroenteritis. J Clin Virol 2012;53:290–6.

Logan C, O'Leary JJ, O'Sallivan N. Real-time reverse transcription PCR detection of norovirus, sapovirus and astrovirus as causative agents of acute viral gastroenteritis. J Virol Method 2007;146:36–44.

Lu X, Holloway B, Dare RK, Kuypers J, Yagi S, Williams JV, et al. Real-time RT-PCR assay for comprehensive detection of human rhinoviruses. J Clin Microbiol 2008;46:533–9.

Medici MC, Tummolo F, Albonetti V, Abelli LA, Chezzi C, Calderaro A. Molecular detection and epidemiology of astrovirus, bocavirus and sapovirus in Italian children admitted to hospital with acute gastroenteritis, 2008–2009. J Med Virol 2012;84:643–50.

Nix WA, Maher K, Johansson ES, Niklasson B, Lindberg AM, Pallansch MA, et al. Detection of all known parechoviruses by real-time PCR. J Clin Microbiol 2008;46(8):2519–24.

Rovida F, Campanini G, Sarasini A, Adzasehoun KMG, Piralla A, Baldanti F. Comparison of immunologic and molecular assays for the diagnosis of gastrointestinal viral infections. Diagn Microbiol Infect Dis 2013;75:110–1.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4. Mol Biol Evol 2007;24:1596–9.

Tran A, Talmud D, Lejeune B, Jovenin N, Renois F, Payan C, et al. Prevalence of rotavirus, adenovirus, norovirus and astrovirus infections and coinfections among hospitalized children in northern France. J Clin Microbiol 2010;48(5):1943–6.

Van Doornum GJ, Schutten M, Voermans J, Guldemeester GJJ, Niesters HGM. Development and implementation of real-time nucleic acid amplification for the detection of enterovirus infections in comparison to rapid culture of various clinical specimens. J Med Virol 2007;79:1868–76.

Van Maarseveen NM, Wessels E, De Brouwer CS, Vossen AC, Claas EC. Diagnosis of viral gastroenteritis by simultaneous detection of Adenovirus group F, Astrovirus, Rotavirus group A, Norovirus genogroups I and II, and Sapovirus in two internally controlled multiplex real-time PCR assays. J Clin Virol 2010;49:205–10.

Wilhelmi I, Roman E, Sánchez-Fauquier A. Viruses causing gastroenteritis. Clin Microbiol Infect 2003;9:247–62.

Yamashita T, Sugiyama M, Tsuzuki H, Sakae K, Suzuki Y, Miyazaki Y. Application of a reverse transcription-PCR for identification and differentiation of Aichi Virus, a new member of the picornavirus family associated with gastroenteritis in humans. J Clin Microbiol 2000;38:2955–61.