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Human Parechovirus Infections in Patients Admitted to Hospital in Northern Italy, 2008–2010

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Human parechoviruses (HPeVs) infection is associated with a wide range of clinical syndromes such as respiratory, gastrointestinal, neurologic diseases, and neonatal sepsis-like illness. The main objective of this study was to investigate the epidemiology of HPeVs infection in hospitalized patients in a period of 2 years. Respiratory samples from 3,525 patients with respiratory syndrome, cerebrospinal fluid (CSF) from 340 patients with neurologic syndrome as well as CSF and plasma samples from five neonatal patients with sepsis-like illness collected from October 2008 to 2010 were tested retrospectively using HPeV-specific real-time RT-PCR. Phylogenetic analysis of VP3/VP1 region was performed on the positive samples. Fourteen out of 3,525 (0.4%) patients with respiratory syndrome and five out of five patients with sepsis-like illness were positive for HPeV. In 3/5 patients with sepsis-like illness multiple samples (e.g., stool, plasma, CSF, or respiratory samples) were available, and HPeV was found in all specimens. In contrast, no positive CSF was detected among the 340 patients with neurologic syndromes. Eleven patients (57.9%) were infected with HPeV1 strain, 7 (36.8%) with HPeV3, and 1 (5.3%) with HPeV6 strains. Ten of the 14 HPeV patients with respiratory syndrome were co-infected with other respiratory viruses (eight with rhinovirus and two with coronavirus OC43). All five patients with sepsis-like illness were less than 1 month of age and were infected with HPeV3. Although not circulating at high frequency and unlikely to cause respiratory syndrome, HPeV was associated with severe clinical syndromes in a minority of newborns. ***J. Med. Virol. 84:686–690, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: human parechovirus; respiratory syndromes; sepsis-like illness; newborns

INTRODUCTION

Human parechoviruses (HPeVs) belong to the Picornaviridae family and were previously classified as echovirus 22 and echovirus 23 [Hyypiä et al., 1992; Oberste et al., 1999]. Fourteen HPeV genotypes have been described worldwide [Ito et al., 2004; Boivin et al., 2005; Benschop et al., 2006a, 2008; Al-Sunaidi et al., 2007; Watanabe et al., 2007; Drexler et al., 2009; Li et al., 2009; Calvert et al., 2010; Kim et al., 2010]. Clinical manifestations of HPeV infection including gastrointestinal, respiratory, and neurologic syndrome. HPeV1 and 2 have been associated with mild respiratory and gastrointestinal infections [Abed and Boivin, 2006; Harvala et al., 2008; Harvala and Simmonds, 2009] and, in rare cases, with necrotizing enterocolitis [Birenbaum et al., 1997]. HPeV3 has been associated with sepsis-like illness, severe neurologic manifestations [Ito et al., 2004; Boivin et al., 2005], and infant death [Levorson et al., 2009; Sedmak et al., 2010]. Although HPeV genotypes 4–14 were identified in a number of different countries, their clinical role remains to be clarified [Benschop

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et al., 2006a, 2008; Al-Sunaidi et al., 2007; Watanabe et al., 2007; Drexler et al., 2009; Li et al., 2009; Calvert et al., 2010; Kim et al., 2010]. On the other hand, HPeVs are not included usually in diagnostic panels, thus, the HPeV infection rate might be underestimated.

The aims of present study were to investigate the prevalence of HPeV infections in hospitalized patients in the Northern Italy, analyze the clinical characteristics of HPeV infections, and characterize the circulating strains by phylogenetic analysis.

MATERIALS AND METHODS

In the period October 2008–2010 were tested retrospectively by HPeV-specific real-time RT-PCR [Nix et al., 2008]: (i) 5,283 respiratory samples (nasal swab, nasopharyngeal aspirates, or bronchoalveolar lavage) from 3,525 patients (1,938 pediatric and 1,587 adult) with respiratory syndromes; (ii) 353 cerebrospinal fluid (CSF) from 340 patients with neurologic syndromes (80 pediatric and 260 adult); and (iii) multiple specimens (three CSF, three plasma, five respiratory, and three stool sample) from five neonates with sepsis-like illness. Patients were stratified with respiratory syndrome according to Baumgarte et al. [2008] comparing patients <2 years of age with respect to patients >2 years of age.

All respiratory samples were tested prospectively with a diagnostic panel of 17 respiratory viruses [Piralla et al., 2009]. CSF samples were tested prospectively for herpes simplex, varicella zoster, cytomegalovirus, polyomavirus, and enterovirus. Virologic and clinical data for HPeV-positive patients were retrieved from medical records.

Genotyping was performed by sequencing amplification products of the VP3/VP1 region [Harvala et al., 2008] using the BigDye Terminator cycle sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) and the ABI Prism 3100 DNA sequencer (Applied Biosystems). Sequences were assembled with Sequencer software (4.6 version, Gene Code Corp., Ann Arbor, MI) and multiple sequence alignment was conducted using MEGA version 5.0 [Tamura et al., 2011]. Nucleotide sequences from the VP3/VP1 region have been submitted to GenBank under accession numbers JN112318-JN112338.

RESULTS

Fourteen out of 3,525 (0.4%) patients with respiratory syndromes and five out of five patients with sepsis-like illness were positive for HPeV (Table I). None of the patients with neurologic syndromes was positive for HPeV. Of the 14 HPeV-positive patients with respiratory syndromes 6 (42.9%) had lower respiratory tract infections, while 8 (57.1%) had upper respiratory tract infections. In two premature patients (#11, and #13, born at 25 and 29 weeks, respectively) with sequential respiratory samples HPeV-RNA persisted for up to 25 days (Table I). The number of patients <2 years of age (n = 831) was comparable with that of patients >2 years of age (n = 1,107). Considering the detection rate of respiratory syndrome caused by HPeV only in patients <2 years the rate increased to 1.6% (13/831) with respect to 0.4% of total rate.

The patients with sepsis-like illness were younger (median age, 17 days) with respect to the patients with respiratory infections (median age, 365 days; $P < 0.01$). Among the patients with sepsis-like illness, HPeV was detected in different clinical specimens in three patients (#15, #18, and #19, Table I). Despite neurologic involvement, in two other patients (#17 and #19) CSF samples were not available and HPeV was detected in plasma samples. HPeV3 infection was community acquired in four patients with sepsis-like illness (#7, #15, #17, and #19), while in one patient (#18) nosocomial infection was observed. All patients recovered from the infection.

HPeV-positive patients were identified during the entire study period, 6 (31.6%) in 2008, 7 (36.8%) in 2009, and 6 (31.6%) in 2010. HPeV infections peaked during the fall season with 14/19 (73.7%) positive patients, followed by 5/19 (26.3%) in summer and 1/19 (5.3%) in spring. Ten of 14 (71.4%) HPeV-positive patients with respiratory syndromes were co-infected with other respiratory viruses (eight with rhinovirus and two with coronavirus OC43). In five patients with sepsis-like illness no coinfecting viruses were found.

Eleven of 19 (57.9%) patients were infected with an HPeV1 virus strain, 7/19 (36.8%) patients were infected with an HPeV3 virus strain, and the remaining patient was infected with an HPeV6 virus strain (Fig. 1).

Nucleotide identity among HPeV1 sequence strains ranged from 84.3% to 100%, and in HPeV3 sequences from 92.9% to 100%. HPeV1 sequence strains from patients #11, #12, and #13 were closely related (100% identity) and were indentified in patients hospitalized in the same pediatric unit (Table I). The HPeV3 sequences amplified from different clinical samples of patient #18 were identical to each other. HPeV3 sequence from patient #6 was amplified from a NPA sample collected in 2008 and was divergent from sequences circulating in 2009 and 2010.

DISCUSSION

In agreement with previous data a low prevalence of HPeV in respiratory samples was observed [Harvala et al., 2008] and detection was restricted to the patients <2 years of age. The frequent association of HPeV infections with other respiratory viruses may indicate a less pathogenic role for HPeV compared to the other viruses infecting the respiratory tract. The low number of single infections in our series did not allow to draw major conclusions on the role of HPeV respiratory syndromes. Few data have been published on the duration of virus shedding. In our series, HPeV infection persisted for 25 days in two neonates.

TABLE I. Clinical Manifestation of HPeV Infections

Patient no.	Sex/age (years)	Date sample collection (month/year)	Positive clinical samples	Days follow-up	Symptoms	Others signs or symptoms	Underlying disease	HPeV typing	Coinfecting virus
1	M/14 mos	10/2008	NPA		Pharyngitis, cough, pneumonia	Leukocytosis		HPeV1	HRV
2	M/11 mos	11/2008	NPA		Fever, cough, dyspnea	CRP: 1.7 mg/dl		HPeV1	HRV
3	M/1	11/2008	NPA		Rhinorrhea, cough	Leukocytosis		HPeV1	hCoV-OC43
4	F/3	11/2008	NPA		Fever, pharyngitis	Leucosytosis, CRP: 7.99 mg/dl otalgy, fever convulsion		HPeV6	hCoV-OC43
5	M/1	11/2008	NPA		Rhinorrhea, cough	Otalgy, CRP: 2,75 mg/dl, previous athmatic episode		HPeV1	
6	M/30	12/2008	NPA		Rhinorrhea		Lung transplant (2005)	HPeV3	HRV
7	F/29 days	07/2009	CSF		Fever, sepsis-like illness			HPeV3	
8	M/1	08/2009	NPA		Fever, cough, wheezing, pneumonia	Leukocytosis		HPeV3	
9	M/6 mos	11/2009	NS		Rhinorrhea			HPeV1	
10	F/13 mos	11/2009	NS		Rhinorrhea			HPeV1	
11	F/2 mos	12/2009	NS	0	Cough		Premature (25 weeks), CPAP	HPeV1	HRV
			NS	11	Cough				
			NS	19	Cough				
			NS	26					
12	F/50 days	12/2009	NPA		Rhinorrhea, cough, diarrhea			HPeV1	HRV
13	F/30 days	12/2009	NPA	0	Rhinorrhea, cough, pneumonia		Premature (29 weeks)	HPeV1	HRV
			NPA	16			Bronchopolmunary displasy		
			NPA	29					
14	F/17 mos	04/2010	NS		Fever, wheezing, dyspnea	Leukocytosis, saturation O_2: 90%. CRP: 5.35 mg/dl, previous asthmatic episodes		HPeV1	HRV
15	F/1 mos	07/2010	CSF, NS, Plasma		Fever, sepsis-like illness	Diarrhea, otitis		HPeV3	
16	M/2	09/2010	NS		Fever, cough, cough, dyspnea	Saturation O_2: 96%, previous asthmatic episodes		HPeV1	HRV
17	M/14 days	09/2010	Plasma		Sepsis-like illness, encephalitis			HPeV3	
18	F/11 days	10/2010	CSF, NPA, stool		Sepsis-like illness			HPeV3	
19	M/17 days	10/2010	Plasma, PS		Sepsis-like illness			HPeV3	

NPA, nasopharyngeal aspirate; CSF, cerebrospinal fluid; NS, nasal swab; PS, pharyngeal swab; CRP, C reactive protein; HPeV, human parechovirus; HRV, human rhinovirus; hCoV, human coronavirus; mo, month; NA, not available; CPAP, continuous positive airway pressure.

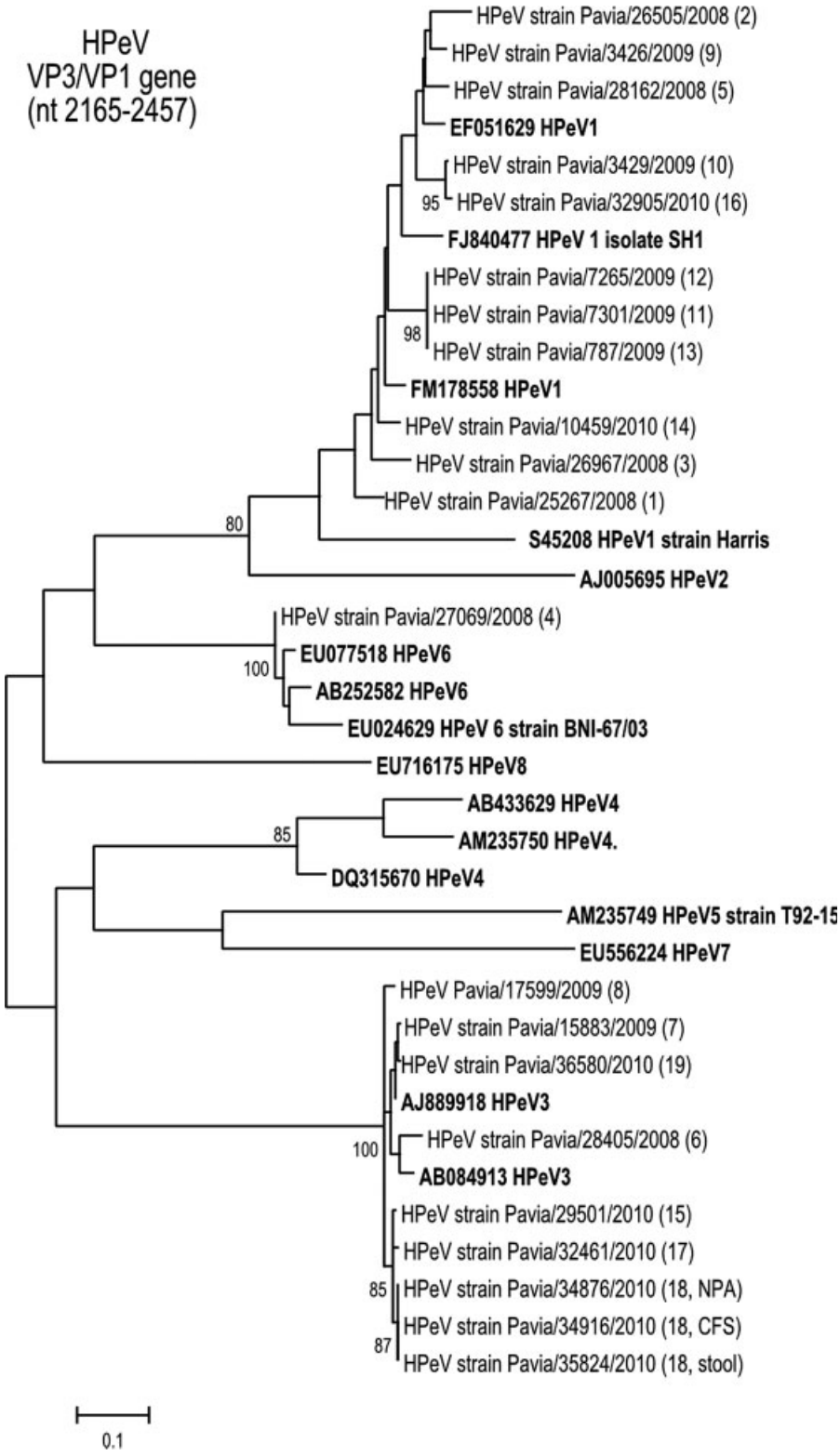

Fig. 1. Phylogenetic tree constructed with VP3/VP1 (nt 2,165–2,457 with respect to HPeV1 strain Harris; accession number S45208) nucleotide sequences from reference strains (in bold) and Italian HPeV strains (n = 21) from 19 patients. Phylogenetic analysis was inferred by using the maximum likelihood method based on the Tamura 3-parameter model as an evolutionary model. A discrete gamma distribution was used to model evolutionary rate differences among sites. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site.

HPeV3 has been shown recently to be an important cause of severe infections in very young children including sepsis-like illness and encephalitis [Ito et al., 2004; Boivin et al., 2005; Levorson et al., 2009]. In our series, five patients <1 month of age with sepsis-like illness were infected with HPeV3. In three of these patients, multiple samples (plasma, stool, CSF, or respiratory sample) were HPeV-positive. These findings support the hypothesis of a disseminated infection caused by HPeV3 [Piñeiro et al., 2010; Sedmak et al., 2010]. The detection of HPeV in different biologic specimens from patients with sepsis-like illness may help to clarify the extent of the infection. On the other hand, none of patients with neurologic syndromes in the absence of sepsis-like illness was positive for HPeV, thus suggesting that HPeV neurotropism might be limited to specific clinical conditions. In keeping with other reports [Benschop et al., 2006b, 2008; van der Sanden et al., 2008], the majority of HPeV infections in our study were observed in the fall season.

Despite the limitations of this retrospective study and the need for prospective studies to better define the clinical impact of HPeV infection, some conclusions can be drawn: (i) HPeV does not circulate with high frequency, (ii) the role of HPeV in the respiratory syndrome indicated a bystander phenomena; (iii) HPeV may be associated with severe clinical syndromes in a minority of newborns, and (iv) detection of HPeVs in different clinical samples should always be considered in newborns with sepsis-like illness.

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