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Clinical Characteristics of Human Parechoviruses 4-6 Infections in Young Children

**Dasja Pajkrt¹, Kimberley S.M. Benschop², Brenda
Westerhuis², Richard Molenkamp², Louise Spanjerberg³,
Katja C. Wolthers²**

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¹ Dept. of Pediatric Infectious Diseases, Emma Children's Hospital,
Academic Medical Center, Amsterdam.

² Lab. of Clinical Virology, Dept. of Medical Microbiology, Academic Medical
Center, Amsterdam.

³ Dept. of Pediatrics, Amstelland Hospital, Amstelveen.

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Background. Human parechoviruses (HPeVs) and enteroviruses (EVs) belong to the family *Picornaviridae*. EVs are known to elicit a wide range of disease such as meningitis, encephalitis, and sepsis. HPeV1 and 2 have been associated with mild gastrointestinal or respiratory symptoms in young children. HPeV3 is associated with more severe neonatal infection. Little is known about the epidemiology and pathology of HPeV4-6 in children.

Methods. We evaluated the clinical symptoms of the children with an HPeV 4, 5 or 6 infection. The patients with positive HPeV 4-6 in stool samples were selected and available plasma or cerebrospinal fluid samples from these patients were tested for HPeV. Data on clinical symptoms, diagnosis, presence and duration of fever, medical history, mean age, use of antibiotics of the children infected with HPeV4-6 were retrospectively documented.

Results. HPeV 4-6 were found in 31 of the 277 HPeV positive children (11%). Co-infection with EV was seen in 8 patients. Fever was seen in 13 (42%) patients. Of the HPeV 4-6 positive patients, 20 of the 31 children (64%) presented with gastrointestinal complaints and 18 of 31 (58%) patients had respiratory symptoms. The mean age was 11 months, 58% of the patients had an underlying disorder such as bronchomalacia or a cardiac disorder.

Conclusions: Symptomatic HPeV4-6 infections are seen in relative young children and are associated with respiratory and/or gastrointestinal symptoms. HPeV type 4 was detected more frequently than HPeV types 5 and 6.

INTRODUCTION

Human Parechoviruses (HPeV) and Enteroviruses (EVs) belong to the family *Picornaviridae*. EVs are well established causes of a variety of diseases such as sepsis, encephalitis and meningitis in children [1], but studies on the clinical pathology of HPeV infections are scarce. HPeV type 1 and 2 were previously known as echovirus 22 and echovirus 23 [2], and are associated with respiratory tract and gastrointestinal infections, and with otitis media in young children [3-6]. Encephalitis [7,8] and paralysis [9] have also been associated with a HPeV1 infection. HPeV types 3, 4, 5 and 6 have only recently been discovered [10-13] and little is known of the clinical pathology

associated with infection by these viruses. HPeV3 has been associated with more severe disease such as neonatal sepsis and meningitis [10, 14,15]. Most recently, encephalitis with white matter injury caused by HPeV was described in ten neonates [16]. HPeV genotyping directly from CSF showed that all HPeVs that could be typed (80%) were HPeV3. HPeV4 was first identified in an infant with fever and feeding problems [11]. HPeV6 was isolated from a child with Reye's syndrome and subsequently 10 children with gastroenteritis, respiratory symptoms and a rash were found to be infected with HPeV6 as was also a child with flaccid paralysis [13].

In clinical practice diagnosing EV in a patient clinically suspected of sepsis or meningitis is accomplished by RT-RT-PCR using the conserved 5' Untranslated Region (UTR) [2,17,18]. Because of distinct sequences at the 5' end in the EV and HPeV genes [19,20], HPeV will not be detected with these molecular assays for EV. We have recently developed a real time Taqman RT-PCR assay directed at the 5'UTR to detect HPeV directly from clinical samples [18]. For genotyping, the VP1 region is sequenced directly from stool samples [21].

This method was used to study retrospectively the prevalence of HPeV 4-6 in fecal samples from children younger than 5 years of age obtained from 2004 until 2007 and clinical characteristics of the children infected with HPeV 4-6 were documented. Additionally, from patients with positive HPeV 4-6 feces samples, available samples from plasma, cerebrospinal fluid (CSF) and nasopharyngeal aspirates (NPA) were tested for HPeV presence.

METHODS

Detection and genotyping of HPeV by real-time RT-PCR

From 2004 until 2007, feces samples that had been referred to the Laboratory of Clinical Virology for viral diagnostics were routinely stored at -80°C. Samples ($n=2,372$), obtained between 2004 and 2007 from children <5 years ($n=1,809$) were respectively screened using an HPeV specific real time RT-PCR [5].

Positive HPeV samples were genotyped by sequencing the complete VP1 region as previously described [21].

From patients that had positive HPeV 4-6 detection in the feces, additional HPeV testing of available materials from ethylenediaminetetraacetic acid (EDTA) plasma ($n=5$), NPA ($n=5$) and CSF ($n=4$) was performed as described before [18,22].

Furthermore all available materials from patients with positive HPEV4-6 feces samples were tested for EV presence according to previously published RT-PCR method [18].

Clinical data

The clinical characteristics of children with positive HPeV4-6 in the fecal samples were obtained using a questionnaire. The following items were retrospectively registered using the medical records or letters of discharge: age at time of virus isolation, sex, medical history (including prematurity) and length of hospital stay. The presence and duration of fever (temperature $>38^{\circ}\text{C}$), sepsis-like illness (fever or hypothermia with signs of circulatory and/or respiratory dysfunction defined by tachycardia or bradycardia, low blood pressure and decreased saturation), neurologic symptoms (meningitis with elevated CSF cell count (> 10 cells/ mm^3), with or without raised CSF protein level (>0.35 g/L) or decreased CSF glucose level (< 2.8 mmol/L), irritability, encephalitis, seizures, or paralysis were documented. In addition, respiratory symptoms (rhinorrhoea, cough, tachypnea, inter- or subcostal retractions, wheezing, inspiratory stridor, and abnormalities on chest radiograph), otitis and gastrointestinal symptoms (diarrhea, nausea and/or vomiting), rashes, use of antibiotics and diagnosis at discharge were recorded. If a specific symptom was not clearly mentioned in the medical record or letter of discharge, the symptom was labeled as 'missing'.

RESULTS

HPeV samples

Between 2004 and 2007, all fecal samples of children younger than 5 years were tested for HPeV by RT-PCR ($n=2,372$), and 277 children tested positive. Of all the HPeV positive samples, 31 patients were diagnosed with HPeV4-6 (11%), 20 patients were positive for HPeV genotype 4, six patients with HPeV5 and five with HPeV6 (Table 1). In only two patients HPeV was detected in plasma and NPA. Of the 31 patients, 8 children had positive EV feces samples at the same time (five HPeV4 patients, two HPeV5 and one HPeV6 patient). Only one patient, that is described in more detail below, had a positive EV infection in plasma. There was no relation between the detection of EV and the occurrence of fever, gastro-intestinal or respiratory symptoms. There were 22 boys and 9 girls. The mean age of the children was 11 months (median 10 months) There were two neonates of two weeks old. More than half of the patients (58%) had an underlying illness and 22% of the patients was born prematurely. In 26% ($n=8$) of the patients another

illness was present besides the HPeV infection. The mean duration of hospital stay was 8 days (median, 4 days) and 55% of the children were given antibiotics for an average of 5 days. All but one patients were admitted: 9 to the general hospital, and 21 children to the academic hospital, of whom 5 to the intensive care unit.

Clinical characteristics of children with HPeV4-6 in feces

Clinical data of the children tested positive for HPeV4-6 in feces were available from all children (Table 2). Fever was present in 13 (42%) children with a mean duration of 3 days. There was only one patient with signs of a sepsis-like illness. In 2 children meningitis (with 1552 and 91 cells/3 μ l in CSF respectively and protein levels of 2.28 and 0.80 g/l respectively) was diagnosed. The first of the two patients had a medical history of hydrocephalus with a ventriculo-peritoneal shunt. The CSF of this child was tested on herpes simplex virus, EV, HPeV by RT-PCR and a bacterial culture of the CSF was performed; no viral or bacterial pathogen was detected. In the other child *S. pneumoniae* was cultured from the CSF.

Table 1. Baseline characteristics of 31 patients with HPeV4-6 in fecal samples.

Variable	Finding
Sex males/females	22/9
Age, months	
Mean	11
Median (range)	10 (3-64)
Underlying condition	18 (58)
Prematurity	7 (22)
Other acute illness at time of HPeV infection	8 (26)
Hospital stay, days	
Mean	8
Median (range)	4 (0-40)
Admission to general hospital	9 (29)
Admission to academic hospital	21 (68)
Admission on IC unit with artificial ventilation	5 (16)
Use of antibiotics	17 (55)
Duration of antibiotic treatment, days	
Mean	5
Median (range)	7 (3-14)
HPeV 4	20 (65)
HPeV 5	6 (19)
HPeV 6	5 (16)
EV	8 (25)

Note: Data are amount followed by (%) of patients with symptom, unless stated otherwise. IC (Intensive Care), HPeV (human parechovirus), EV (enterovirus).

Table 2. Clinical data of 31 patients with HPeV4-6 in fecal samples.

Variable	Finding
Fever	13 (42)
Duration of fever, days	
Mean	3
Median (range)	2,5 (1-7)
Sepsislike illness	1 (3)
Meningitis	2 (7)
GI tract symptoms	20 (64)
Diarrhea	18 (58)
Duration of diarrhea, days	
Mean	4
Median (range)	2,5 (1-21)
Respiratory tract symptoms	18 (58)
Coughing	7 (23)
Rhinitis	13 (42)
Sub-or intercostal retractions	5 (16)
Wheezing	1 (3)
Pneumonia	6 (19)
Otitis	6 (19)
Rash	3 (10)

Note: Data are amount followed by (%) of patients with symptom, unless stated otherwise.

In 20 children gastrointestinal symptoms, 18 with diarrhea were present, with a mean duration of 4 days. In 58 percent of the patients respiratory symptoms such as rhinorrhea, pneumonia, retractions or wheezing were present. In 6 patients otitis media was diagnosed. A rash was noted in 3 patients.

In table 3 the detection of HPeV4 and EV from different samples is depicted together with the clinical symptoms of one patient. The patient was a prematurely born boy with a omphalocele and bronchopulmonary dysplasia that was hospitalized all his life and that presented with respiratory and gastrointestinal symptoms at the age of 3 months and 11 days. On day 0 HPeV4 was first detected in the NPA and was associated with a pneumonia followed by recovery from feces on day 3. An increase in respiratory symptoms coincided with the presence of HPeV4 in plasma, a pneumonia was simultaneously diagnosed at this time point. Subsequently the patient was infected with EV as was demonstrated by the detection of EV in plasma and feces. Simultaneously with a decrease in clinical symptoms, EV and HPeV4 were no longer detected. Both HPeV4 and EV could be detected from feces samples up to 40 days after the initial detection.

Table 3. Relation of clinical symptoms and detection of HPeV4 in one patient.

Day	0	3	6	23	27	29	43	69	93
GI symptoms	Recurrent gastroenteritis								
Respiratory symptoms	pneumonia			pneumonia			Recurrent URTI		
Feces		HPeV+		HPeV+		HPeV+	HPeV+	HPeV-	HPeV-
		EV-		EV-		EV+	EV+	EV+	EV-
Nasopharyngeal aspirate	HPeV+			HPeV+					
	EV-			EV-					
plasma			HPeV-		HPeV+		HPeV-		HPeV-
			EV-		EV+		EV+		EV-

On day = 0 HPeV4 could be detected from NPA for the first time (patient was 3 months and 11 days old). At time points where there are no results depicted, there were no samples available for testing. GI= gastrointestinal, URTI= upper respiratory tract infection

DISCUSSION

In this study we describe for the first time the characteristics and clinical symptoms of young children with an HPeV4-6 infection. The majority of the children were boys, a finding that is seen before in studies on HPeV [22]. More than half of the patients had an underlying illness, which suggests that HPeV4-6 could cause more severe illness in these children in comparison to previously healthy children.

The mean duration of hospital stay for children with HPeV4-6 in feces was 8 days and antibiotic treatment was given to more than half of the cases. It has been demonstrated that reduction in hospital stay and antibiotic treatment can be achieved by rapid diagnostic testing for EV [23], but data involving rapid diagnostic tests for HPEV for hospital stay reduction and antibiotic use are lacking.

In 25% of the patients with positive HPeV4-6 in feces, EV could be detected in plasma, feces or NPA. As the questionnaires did not contain a severity scale for the clinical signs, we do not know whether a dual infection with HPeV and EV elicited more severe symptoms in comparison with only an EV or HPeV infection.

The majority of the children with a HPeV4-6 infections had gastrointestinal or respiratory symptoms. Interestingly, the 6 cases of otitis media were associated with all the three different types studied on this study (3 patients with HPeV4, 2 patients with HPeV5 and 1 patient with HPeV6). Otitis media has also been associated in children infected with HPeV1 [6]. Additional studies are needed to determine whether HPeV4-6 are causally associated with otitis media in childhood. Sepsis or sepsis-like illness was diagnosed in only one of the children and although two patients had clinical signs of a meningitis and increased CSF cell count and CSF protein value, HPEV could not be detected in the CSF of these children. It is well known that EV can cause sepsis-like illness and aseptic meningitis in young children [1,24] and recently we demonstrated that patients with HPeV in the CSF presented with sepsis-like illness and meningitis [22]. HPeV3 infections are associated with more severe disease such as neonatal sepsis and meningitis, even when isolated only from stool samples [14,15]. Patients with a HPeV1 and HPeV2 infection present mainly with mild gastrointestinal or respiratory complaints [25,26] and our data suggest that HPeV4-6 can be associated with similar symptoms. A limitation of our study is that the sample collection is biased as they derive from a pediatric population referred for virologic diagnostic testing. We did not include a control group, so it is unknown whether asymptomatic shedding of HPeV4-6 in feces occurred in our study. In a

recent study, HPeV6 was found in 9% of non-hospitalized children without causing any illness whereas HPeV4 and HPeV5 were not detected in this study [27].

There are no reports on the possible duration of excretion of HPeV in fecal samples. One study showed the duration of enteroviruses to last up to 11 weeks after infection [28]. Here we report one case of a young infant infected with HPeV4 followed by EV. Both viruses could be detected in various samples for as long as 40 days. In this one case detection of HPeV4 and EV was associated with clinical symptoms at all testing times, but a clear contribution of HPeV4 or EV cannot be given.

In summary, we conclude that HPeV4-6 are associated with gastrointestinal and respiratory symptoms in young infants. In our study, HPeV4-6 infections are not associated with sepsis-like illness or aseptic meningitis.

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