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CASE REPORT

Parechovirus infection causing sepsis-like illness in newborns: a NICU approach

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SUMMARY

Human parechovirus (HpeV) is an important emerging infection in young infants, able to cause sepsis-like disease and meningoencephalitis, especially in newborns. Among the 19 identified genotypes, HPeV1, 3 and 6 are the most common types involved in human infections; HPeV3 is the type mainly responsible for neonatal infections and for infections involving the central nervous system. Signs and symptoms overlap with those of a bacterial infection and patients are usually treated with broad spectrum antibiotics. In the majority of cases lumbar puncture shows absence of pleocytosis, even in the presence of signs of meningitis. In these cases, cerebrospinal fluid cultures are negative for bacteria but, in the absence of diagnosis of viral infection, a full and unnecessary antibiotic cycle is often continued. Moreover, high sensitivity neuroimaging, i.e., magnetic resonance, and follow-up are often missed, thus resulting in substandard care. Availability of a real time PCR assay for HPeV RNA allows rapid and sensitive diagnosis as long as the disease is suspected. In this case study, we present cases of HPeV infections in newborns requiring neonatal intensive care admission, discuss their optimal management, and highlight the most relevant findings in the literature.

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INTRODUCTION

Human parechoviruses (HPeVs) are single-stranded RNA viruses belonging to the Picornaviridae family; they were formerly included in the Enterovirus (EV) genus and only in the 1990s were reclassified into their own genus (Hyypia *et al.,* 1992). HPeV is a common cause of infections occurring during the first years of life, with a broad spectrum of manifestations ranging from a complete absence of symptoms to a sepsis-like disease, especially in newborns, that can require intensive care assistance (Kadambari *et al.,* 2019; Britton *et al.,* 2018; Olijve *et al.,* 2017; de Crom *et al.,* 2016; Esposito *et al.,* 2014). Nowadays, 19 different types are recognized (HPeV1 to 19), but human diseases are associated mostly with genotypes 1, 3 and 6. HPeV1 tends to cause mild gastrointestinal symptoms in older children; instead, genotype HPeV3 is almost invariably found in newborns, causing a sepsis-like illness and conditions involving the central nervous system (CNS) (Kadambari *et al.,* 2019). This infection has been defined as an important emerging infection in young infants, therefore, greater awareness of this disease is required among neonatologists, with implementation of protocols for diagnosis and management (Britton *et al.,* 2018).

We describe 6 cases of HPeV infection in newborns requiring neonatal intensive care unit (NICU) admission and discuss the approach to improved diagnosis and management of HPeV-affected newborns.

Key words:
Human parechovirus, sepsis-like illness, meningoencephalitis, newborn, RT-PCR.

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CASE REPORTS

Demographics and clinical characteristics of the patients are presented in *Table 1*. All but one of the infants were born at term: one infant was born at 33+4 weeks gestational age. Median age at presentation was 13 days (range 5-31 days). All presented with fever, ranging from mild alteration to 40°C, and poor feeding; four also had irritability, one showed neurological signs. Blood exams performed at the time of the disease onset showed a median white blood cell count of 6,715/mmc (range 3,540-8,750) and in all cases a lymphopenia was detected (median value 1,169/mmc, range 730-1,406). None had thrombocytopenia. Serum C-reactive protein (CRP) was normal or only slightly increased in all patients (median value 0.78 mg/dl, range 0.17-1.34), with a median maximum value during the course of the disease of 0.86 mg/dl (range 0.66-2.58). Cerebrospinal fluid (CSF) examination did not show pleocytosis in any infant (median white cell count 4/mmc, range 0-5). Protein and CSF-to-serum glucose ratio were in the normal range for age (*Table 2*).

In all patients CSF samples were collected in sterile con-

Table 1 - *Demographic and clinical characteristics.*

Patient	*Sex*	*GA at birth (wks)*	*Season at presentation*	*Family cluster*	*Symptoms at presentation*	*Highest temperature (°C)*	*Max heart rate (bpm)*	*Other signs and symptoms*
1	F	40+4	Fall	Yes	Fever, poor feeding, irritability	40	200	Rash, pericardial effusion
2	M	33+4	Summer	Yes	Fever, poor feeding, seizures	37.5	200	Respiratory insufficiency, shock, pericardial effusion meningoencephalitis
3	M	40	Summer	Yes	Fever, poor feeding, irritability	40	170	Rash, diarrhea
4	M	38+1	Fall	Yes	Fever, poor feeding	38	160	Rash
5	F	40+1	Summer	Yes	Fever, poor feeding, irritability	38.2	230	Shock, pericardial effusion
6	F	40	Summer	Not known	Fever, poor feeding, irritability	38	210	Seizures

GA: gestational age.

Table 2 - *Cerebrospinal fluid findings.*

Patient	*Aspect*	*Glucose (mg/dl)*	*Protein (mg/dl)*	*White blood cell count/mmc*	*Mononuclear Cells (%)*
1	clear	60	40	5	80
2	clear	63	57	4	75
3	clear	58	50	4	75
4	clear	44	80	3	77
5	clear	77	43	4	75
6	clear	53	60	0	/

tainers without viral transport media. In 4 patients whole blood samples were also collected in EDTA-anticoagulated tubes and urine and stool specimens in sterile containers without viral transport media.

The qualitative detection of HPeV RNA in whole blood, urine and stool samples was performed by using a commercially available one-step, reverse-transcription and real-time PCR multiplex assay (Meningitis Viral 2 ELITe MGB® Panel; ELITech Group, Italy). Before extraction, 0.1 gr of stool was transferred into a tube containing 1 mL of phosphate-buffered saline. The tube was vortexed for 1 minute and the resulting suspension was subjected to two consecutive centrifuges (10 minutes at 2500-3000 RPM each); an aliquot of 200 µL of the obtained supernatant represented the input volume for the nucleic acids extraction run. Extraction, amplification and detection of the nucleic acids as well as result analysis were performed with the fully automated ELITeInGenius® instrument (ELITech Group, Italy). Nucleic acids were extracted from 200 µL of each sample type and eluted in 100 µL. An aliquot of 10 µL of each sample of body fluids extracted was processed for the amplification run. The fully automated multiplex PCR test, FilmArray™ Meningitis/Encephalitis Panel (BioFire Diagnostics LLC, Salt Lake City, UT - a bioMerieux Company), requires 200 µL of cerebrospinal fluid and was performed following the manufacturer's instructions (Piccirilli *et al.,* 2018).

All tested biological specimens were positive for HPeV RNA. In all infants, CSF and blood samples were negative for bacteria and fungi cultures and for Enteroviruses and Herpetic viruses. All infants underwent echocardiography, which revealed a mild pericardial effusion in three cases, without evidence of myocardial involvement despite the presence of tachycardia disproportionate to fever in three infants. In addition, a palmar-plantar erythema appeared in three infants between day 2 and day 4 of disease, and lasted for 24-48 hours. The course of the disease was severe in two infants, who required mechanical ventilation and inotropic support. One of these two babies was born preterm and his general and neurological conditions were severely compromised, consistent with a meningoencephalitis, with lethargy, areflexia, hypotonia, and clinical and electroencephalographic seizure activity, requiring intensive support for 7 days. A single seizure was also demonstrated in another infant with hyponatremia (123 mEq/L) during the course of the disease. Cranial ultrasound (CUS), performed in all infants, and magnetic resonance imaging (MRI), performed in 5 out of 6 infants, were abnormal only in the preterm infant, who had evidence of bilateral porencephalic cysts in a condition of widespread cytotoxic cerebral damage, also involving corpus callosum and corticospinal tracts. Median duration of fever was 3.5 days (range 1-6) and median duration of hospitalization was 11 days (range 7-26). All neonates gradually recovered and all survived. At a median follow-up age of 24 months (range 9-36) all but one are developing well. The preterm baby with neonatal HPeV-related meningoencephalitis developed epileptic encephalopathy, vision impairment and a marked delay in psychomotor development.

DISCUSSION

In this study, we report 6 cases of NICU admissions of newborns with a sepsis-like illness in which HPeV RNA was detected by RT-PCR in CSF samples. All infants presented with fever and poor feeding; irritability or neurological signs were present in all but one newborn. Most of these infants had been in close contact with another infected family member showing gastroenteritis or upper airways illness. In line with previous studies, all cases occurred

during summer or fall (Tang et al., 2016; Cabreizo *et al.,* 2015).

HPeVs have recently been recognized to cause sepsis-like illness with possible CNS involvement, particularly among young children (Olijve *et al.,* 2017). A national laboratory surveillance study in Denmark (Fisher *et al.,* 2014) evaluated 4808 children less than 5 years of age with symptoms consistent with viral infection and documented HPeV positivity in biological specimens in 3% of cases; children with HPeV3 infection were significantly younger than those with HPeV1 infection (median age 37 days versus 199 days) (Fisher *et al.,* 2014). Ninety-four infants less than 3 months of age developed an HPeV infection during an outbreak in Australia: in all of them the HPeV3 genotype was detected (Cumming *et al.,* 2015; Khatami *et al.,* 2015). A study conducted in the UK reported a remarkable and increased frequency, compared with a previous surveillance, of HPeV infections in children less than 3 months of age presenting with sepsis-like illness (Harvala *et al.,* 2011). Authors speculated that not only the greater sensitivity of diagnostic test, but also a change in the viral biological properties and a subsequent higher susceptibility of the population could explain those findings. Indeed, a study estimated that a common ancestor for the HPeV3 type appeared in the human population as recently as 1987 (Calvert *et al.,* 2010). HPeV3 may therefore not have spread sufficiently into adult human populations to provide the essential levels of maternal protection required to prevent neonatal disease. Recently, an emerging HPeV5, causing sepsis like illness, has been identified in Australia (Chamings *et al.,* 2019). Leber *et al.* (2016) evaluated 299 CSF samples from infants less than 2 months of age and found 56/299 cases of viral meningitis, twelve of which were positive for HPeV.

HPeV transmission occurs mainly through the fecal-oral route, but transmission through the respiratory tract has also been suggested (de Crom *et al.,* 2016; Wildenbeest *et al.,* 2010). In our cases HPeV RNA was isolated in all the biological specimens, including in stools. Stool testing has a high sensitivity for HPeV detection (Khatami *et al.,* 2015; de Crom *et al.,* 2013), and it has been described that HPeV may be detected for up to 2 months after infection in some cases. This notion should be kept in mind in NICU to avoid nosocomial transmission and outbreaks of HPeV, even in the absence of gastrointestinal symptoms.

HPeV infection causes a wide spectrum of clinical manifestations in the newborn, most commonly fever, irritability, poor feeding, tachycardia, rash, sepsis like syndrome and severe neurological symptoms (i.e., seizures); the need for intensive care support has also been reported (*Britton et al.,* 2018; Olijve *et al.,* 2017). The high degree of HPeV cytopathic effect, the rapid HPeV replication in neuronal cells, especially of the HPeV3 strain, together with the possible lack of maternal protective antibodies and the immaturity of the immune system in newborns (Harvala *et al.,* 2011; Park *et al.,* 2019), can all explain the high risk of hospitalization and intensive care requirement in young infants (less than 3 months of age), especially in newborns.

In our case series, 2 out of 6 newborns required mechanical ventilation and inotropic support to treat early signs of warm shock. A rash appeared during the course of the disease in 3 infants: it has been reported that a rash is a hallmark of the HPeV infection and infected infants have been described as "red, hot, angry babies" (Khatami *et al.,* 2015). Lymphocyte count was below the 5th Percentile Reference Range for age (Christensen *et al.,* 2012) in all of our cases; this finding confirms the literature report of a more severe leukopenia in patients with a more severe course of the disease, i.e., those requiring NICU admission (Kurz *et al.,* 2015). CRP was not-significantly elevated in any case.

Our cases show that in the febrile infant, the combined evaluation of epidemiological data and clinical and laboratory findings, such as lymphopenia, rash and tachycardia unrelated to fever, may assist clinicians in the diagnosis of HPeV infection. In these cases, meningoencephalitis may manifest with seizure activity, including central apnea, and focal neurological signs. Diagnosis of CNS viral infections has been improved by the advent of new molecular diagnostic technologies such as RT-PCR: its sensitivity exceeds that of cultural techniques (Leber *et al.,* 2016). HPeV is not detected by RNA testing for EV, but a specific PCR is required and these tests are not generally available in all microbiology laboratories (Leber *et al.,* 2016).

In our case series, RT-PCR detected nucleic acid of HPeV in all CSF samples. Despite HPeV RNA detection in CSF samples, CSF parameters were in the normal range for age. Previous studies underline that the absence of CSF pleocytosis is a common feature in HPeV meningoencephalitis (Park *et. al,* 2019). It has also been shown that cytokines level in CSF samples from infants with HPeV meningitis are low and similar to levels found in samples from patients with fever but without meningitis (Park *et al.,* 2019). Nonetheless, the detection of HPeV RNA in CSF should be carefully taken into account in the short and long-term management of neonates: a neuroimaging evaluation and a long-term follow-up should be considered mandatory. Even though CUS is widely used in the assessment of neonates with meningitis, it is not sensitive enough to explore mild white matter involvement. Instead, MRI may detect characteristic features of meningoencephalitis, which may also have prognostic utility (De Vries *et al.,* 2019; Britton *et al.* 2016; Kurz *et al.,* 2015). In our case series, three infants presented with a severe disease characterized by cardiopulmonary insufficiency and/or CNS signs (*Table 1*, patients no. 2, 5, 6). MRI showed widespread damage only in one patient (*Table 1*, patient no. 2), who was born preterm and who, at the time of infection, was 37 weeks post-conceptional age. His MRI showed bilateral white matter damage evolving into periventricular cysts, especially at the occipital level, corpus callosum, caudothalamic nucleus and cortico-spinal tracts, similarly to what has been reported in the literature (Sarma *et al.,* 2019). Only this patient developed a poor neurological outcome at 24 months of age. Even with the limitation of a small sample size, these findings appear consistent with prior research reporting a good general prognosis in otherwise healthy infants and a high risk of poor outcome in preterm infants (Kurz *et al,* 2015; de Jong *et al.,* 2017; de Vries *et al.,* 2019; Britton *et al.,* 2016). However, a recent follow-up study, conducted at three years of age in children diagnosed with HPeV infection during the Australian outbreak in 2013-2014, whose original illness at less than 3 months of age was fever without source and irritability (n=11), sepsis-like illness (n=47) or encephalitis (n=3), documented normal neurodevelopment but increased behavioral problems compared with healthy controls (Britton *et al.,* 2020).

The clinical approach to HPeV infections requires strict monitoring and support to vital functions, since a specific treatment is not available. Pleconaril, a viral capsid inhibitor with activity against Picornaviridae, does not seem to be effective against HPeV infections (Kadambari *et al.,*

2019; van de Ven *et al.,* 2011). Intravenous immunoglobulins have been used in some cases, without clear evidence of benefit (Kadambari *et al.,* 2019; Midulla *et al.,* 1976; Bell *et al.,* 1971). Greater understanding of the HPeV replication cycle may help to clarify the best therapeutic approach to these neonates.

A major limitation of our case series is the lack of evaluation of the HPeV genotype. Of the 19 described genotypes, HPeV genotypes 1, 3 and 6 are the most commonly associated with human disease, and more serious diseases have been associated with HPeV3, which shows a high degree of cytopathic effect and rapid replication in neuronal cells. A timely genotyping of HPeV could assist clinicians in establishing a more tailored approach to affected babies. Another limitation is the lack of data on the prevalence of HPeV infection among the febrile infants in our population, to assess the clinical significance of this virus in the neonatal period. Nonetheless, these data support the need to include the HPeV RNA PCR in the diagnostic work-up of the febrile neonates, thus avoiding unnecessary empirical antibiotic treatment in all infants with a sepsis-like syndrome, according to the principles of good antimicrobial stewardship (Britton *et al.,* 2018; Kadambari *et al.,* 2019; Antolin *et al.,* 2018).

In the meantime, it is necessary to collect good-quality data on the natural history of HPeV infection in the neonate and to highlight the importance of a long-term follow-up of these infants, especially of those with clinical and neuroimaging evidence of meningoencephalitis or with clinical conditions that predispose to negative outcome.

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