Human Parechovirus Infection in Neonatal Intensive Care

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Background: Approximately 5–6% of all infective episodes in neonatal intensive care unit (NICU) are of viral origin. Previous studies suggest that human parechovirus (HPeV) infection presents most commonly in term infants, as a sepsis-like syndrome in which meningoencephalitis is prominent. Our aim was to study the infection rate and associated features of HPeV.

Methods: Blood samples were taken from NICU babies older than 48 hours, who were being investigated for late onset sepsis. Clinical and laboratory data were collected at the time of the suspected sepsis episode. Samples were tested using universal primers and probe directed at the 5′-untranslated region of the HPeV genome by reverse transcriptase polymerase chain reaction (RT-PCR). Results were confirmed by electrophoresis and DNA sequencing.

Results: HPeV was detected in 11 of 84 samples (13%). These infants had a mean [interquartile range (IQR)] gestational age of 28.9 (26.9–30.6) weeks and mean birth weight of 1.26 (SD = 0.72) kg. The median day of presentation was 16 (IQR: 11–27). These characteristics were similar to the infants without positive viral detection. Six infants presented with respiratory signs. One infant presented with signs of meningitis. Six of the 11 episodes of HPeV infection occurred during the winter months (December to February). No HPeV positive infants had abnormal findings on their 28-day cranial ultrasound examination.

Conclusions: We found an HPeV infection rate of 13% in infants being tested for late onset sepsis. HPeV should be considered as a possible cause of sepsis-like symptoms in preterm infants.

Key Words: neonatal, late onset infection, human parechovirus

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Infants in neonatal intensive care are at risk of sepsis after the first 48–72 hours.1 Between 10% and 50% preterm and low birth weight infants admitted to the neonatal intensive care unit (NICU) are diagnosed with late onset sepsis.2–4 Blood culture, currently considered the gold standard for diagnosis of neonatal infection, has poor sensitivity (8–73%) and will not detect all pathogens.5,6 Very little is known about nonbacterial pathogens as a cause of late onset infection. In an observational study, the incidence of viral infection was reported as 1% of all NICU admissions with 5–6% of all late onset infections being of viral origin.7

From what little is known the picornoviruses seem the most common viral pathogen to infect newborn infants. The picornaviruses are divided into 5 subclasses such as polioviruses 1–3, coxsackie (A and B), echoviruses and enteroviruses.8 Recently, echoviruses 22 and 23 been reclassified as human parechovirus (HPeV) 1 and 2, respectively. HPeV is now recognized as an import neonatal infection and currently had 16 identified genotypes.9–11 HPeV 3 has been associated most frequently with severe clinical neonatal infection and white matter abnormalities.12,13 Infants with HPeV most commonly have been reported to present with a “sepsis-like illness” with or without symptoms of meningitis.11

The aim of this study was to determine the incidence of HPeV in a group of babies being tested for possible late onset sepsis, many of them were preterm. We compared the clinical characteristics of infants who had virus detected versus infants who did not. The genetic subtypes of the infecting virus were also determined.

METHODS

We conducted a prospective cohort study including infants of all gestational ages, who were older than 48 hours when first suspected as having an episode of sepsis. Written, informed parental consent was obtained within 48 hours of admission to the NICU of the Royal Jubilee Maternity Hospital, Belfast (before the sepsis episode). Ethical approval was granted by the Office of Research Ethics Committees for Northern Ireland (07/NIR01/71).

Patients with confirmed necrotizing enterocolitis (Bell’s Stage II) were excluded from analysis as this inflammatory condition of the gut has considerable overlap in terms of clinical characteristics and may interfere with the classification of sepsis. Infants were independently categorized as being septic using the German National Nosocomial Surveillance Scoring System [NEO-Natal Krankenhaus Infektions Surveillance System (NEO-KISS)].14 NEO-KISS is a well-established surveillance system for nosocomial infection in Germany other European NICUs.15,16

During the study each infant had a cranial ultrasound performed on days 0, 3, 10 and 28 and at term corrected. A senior neonatologist reviewed all scans.

Sampling and Extraction

In eligible patients, blood was collected by venepuncture or from an indwelling vascular catheter following skin decontamination with an alcohol swab or 0.05% aqueous chlorhexidine solution. A blood volume of 0.5 mL was collected in a potassium ethylenediaminetetraacetic acid-coated Vacutainer tubes (Becton Dickson and Company, Franklin Lakes, NJ) for the molecular assay. Samples were stored at 2°C. Extraction of nuclear material took place within 48 hours of collection to prevent the breakdown of nucleic acid. The QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) was used for the manual extraction of the bacterial genetic material from clinical samples and was used according to the manufacturer’s instructions.

HPeV Assay

The assay to detect HPeV was based on that described by Nix et al.17 The primers AN345 (GTAAACSWGCCTCCTGGGSC-CAAAAG) and AN344 (GGCCCGWGRTCAGATCCCATCAGY) and probe AN257c (FAM-CTTGGGTACCTCGWGGGCACT-CTTC-BHQ) (Eurofins MWG Operon, Ebersberg, Germany) were incorporated into a mastermix preparation [Superscript III Platinum

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One-Step Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) system with ROX reference dye; Invitrogen, Carlsbad, CA]. The primers produce a product between 194 bp and 195 bp for the detection of HPeV.17 From each of the clinical sample extracts 2 μL was added to 18 μL of mastermix for each reaction.

HPeV reference samples, stored in tissue culture supernatant fluid and frozen at −80°C were then extracted and used as 4 positive controls. Two microlitres of nuclease-free water were added to 4 of the wells to act as negative controls. All clinical samples were tested using the HPeV RT-PCR assay. Positive samples were confirmed using gel electrophoresis and DNA sequencing (BLAST, http://blast.ncbi.nlm.nih.gov/).

Samples were also tested for enterovirus using a described and evaluated primer and probe combination based on the work of Verstrepen et al.18 Rotbart19 and Lai et al.20 Two assays were used in succession to detect the presence of enterovirus targeted at the 5′-untranslated region and the VP 1 capsid protein.

Analysis
The population demographics are presented as mean [Standard deviation (SD)] or median and interquartile range (IQR). The population characteristics and laboratory results in the virus positive and negative groups were compared using a nonparametric comparison of means (Mann-Whitney U test). Mode of presentation, proportions of blood culture and sepsis positivity between groups were compared by Pearson χ² test.

RESULTS
The study was conducted over an 18-month period beginning in November 2007. In total, 93 episodes of sepsis in 61 babies were sampled and recorded. Nine babies with necrotizing enterocolitis were excluded. After these exclusions 84 samples were analyzed for viral pathogens (Table 1).

HPeV tested positive in 11 patients (13%). The reasons for sepsis screening are described in Table 2. The 11 patients had a mean weight of 1.26 kg and median gestation of 28.9 weeks. These patients presented at a median of 16 days of age. There was no significant difference between the weight, gestation or age at time of sampling between the HPeV positive and negative HPeV patients (Table 1). No infant tested positive for enterovirus.

The HPeV positive patients were also blood culture positive for bacteria in 6 of 11 cases (54.4%). The bacteria recovered were coagulase negative Staphylococcus in 5 cases and Staphylococcus aureus in 1 case. In the HPeV negative patients 37 of the 73 (50.7%) were culture positive (all coagulase negative Staphylococcus). There was no significant difference between the incidences of positive bacterial culture in the 2 groups (P = 0.81). In the infants who were HPeV positive, 5 of 11 (45.5%) were considered to be septic by the NEO-KISS score. In these 5 patients, 2 had a positive blood culture (Table 2). In the infants who were HPeV negative, 19 of 73 (26%) were also considered to be septic by the scoring system (P = 0.18).

Comparison of the presentation for suspected sepsis between the HPeV positive and negative groups is shown in Table 2. There were no significant differences between the groups across the categories. Many of the patients in both groups presented with respiratory signs.

All infants completed the schedule of cranial ultrasound examinations. None of the infants with detected HPeV had any evidence of white matter injury on their term-corrected ultrasound.

Seasonal Positivity of HPeV
HPeV infection occurred through the year with most positive samples (6 of 11) being collected in winter (December to February) compared with any other single season.

Confirmation of HPeV Positivity
The 11 positive samples were confirmed by gel electrophoresis and nucleic acid sequencing. The median (IQR) C_t value of

<table>
<thead>
<tr>
<th>TABLE 1. Patient Characteristics and Sample Timing</th>
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<tr>
<td>HPeV PCR Positive (N = 11)</td>
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<td>---------------------------</td>
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<tr>
<td>Male:Female</td>
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<td>Median Gestation (weeks)</td>
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<td>Mean weight (kg) (SD)</td>
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<td>Age at time of sample (days)</td>
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<td>Median (IQR)</td>
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<th>TABLE 2. The Major System Involved With the Presentation of Suspected Sepsis Episodes When Sampling in the HPeV Positive and Negative Groups are Described</th>
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<tr>
<td>Presentation of Suspected Sepsis Episode</td>
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<tr>
<td>HPeV PCR Positive (N = 11)</td>
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<td>-----------------------------</td>
</tr>
<tr>
<td>Bacteria +ve</td>
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<tr>
<td>Bacteria +ve</td>
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<tr>
<td>Respiratory</td>
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<td>Gastrointestinal</td>
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<td>Neurological</td>
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<td>Change in antibiotics</td>
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<td>Rise in CRP</td>
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<tr>
<td>Other</td>
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<tr>
<td>Total</td>
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<td>NEO-KISS sepsis score positive</td>
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P value across all presentation groups >0.05. NEO-KISS sepsis score compared in bacteria positive and negative patient groups is also displayed.
the real-time output was 29.02 (25.87–30.36). The PCR products were of the expected size (195 bp). Six samples were sequenced for confirmation. These sequences were interpreted with the online DNA sequence identification software (BLAST, http://blast.ncbi.nlm.nih.gov/) as HPeV type 1.

**DISCUSSION**

We describe for the first time the detection of HPeV 1 in a group of preterm infants in neonatal intensive care presenting with what was presumed to be late onset bacterial sepsis. In the total group, 13% of infants had a confirmed HPeV type 1 present on viral PCR testing. Infants with this viral infection presented with symptoms and signs similar to those of systemic bacterial infection. Viral pathophysiology in neonates is poorly described and comprehensive data on the incidence, type and clinical features of noncongenital viral infections are largely lacking.21

The most comprehensive single study of viral incidence over a 12-year period showed that of the 5396 infants (<32 weeks, <1500 g) admitted to neonatal intensive care, 51(1%) had confirmed viral infection.7 The incidence of bacterial infection was 15%. In this population, the pathogens causing infection included (in order of frequency) enterovirus/parechovirus (39%), respiratory syncytial virus (RSV) (29%), rotavirus (10%), cytomegalovirus (6%), adenovirus (4%), parainfluenza (4%), herpes (4%), rhinovirus (2%) and rubella (2%).

In 2 previous studies of parechovirus epidemiology and in comparing HPeV with enterovirus,11 a variety of detection methods (initially culture-based, more recently molecular PCR) were used on a variety of substrates [cerebrospinal fluid (CSF), blood, feces and respiratory tract secretions]. In addition, limited strains (HPeV 1 or 2 only) could be typed. This would suggest that these previous studies may have underestimated the true frequency of these viral infections.7 A strength of our study is that we used the molecular assay that was described by Nix et al17 and could detect all known HPeV genotypes.1,6 This assay has greater sensitivity than culture-based techniques and has the capacity to detect more HPeV viral subtypes.

The presentation of viral infection preterm infants is relatively unknown. Cases of HPeV infection in preterm infants have been reported.10,22,23 Infants may present with a sepsis-like illness, with gastrointestinal and respiratory symptoms, indistinguishable from bacterial infection.19,24 A recent pilot study has explored viral infection mimicking nosocomial sepsis in preterm infants. This study recruited 64 cases of suspected infection and in the majority of cases had no pathogen detected. Nine infants had a pathogen detected, 2 were positive for coagulase negative Staphylococcus, 1 Klebsiella, 1 RSV and 5 for picornaviruses. There was no bacterial-viral coinfection. There was no uniformity of presentation or laboratory markers. These results provide perspective to our findings and confirm the heterogeneous nature of viral presentation22 and its similarity to bacterial infection. In our study, the typical presentation of HPeV in preterm infants was between 2 and 3 weeks of life, with increasing desaturation/apnea and clinical features of a sepsis-like illness. In other studies, infants presenting with HPeV infections were males, mostly term or near term, with respiratory or gastrointestinal symptoms in the second week of life.7,11

HPeV 3 has most recently been associated with more severe disease and infants presents with meningocencephalitis.12,24 Only 1 infant in our study presented with seizures and possible meningitis. This infant had HPeV 1 detected in blood, no pathogen was recovered from CSF. The routine laboratory sample had no bacterial growth and had a low whole cell response. The blood cultures in this infant grew S. aureus. Reports indicate that viral infection with HPeV does not induce a significant inflammatory response in the CSF.15 All HPeV in this study was sequenced as HPeV1.

Viral infections in neonates have been reported on many occasions as outbreak phenomena. The World Health Organization defines an outbreak as the occurrence of cases of disease in excess of what would normally be expected in a defined community. An online database to support outbreak investigations26 has reported that up to January 31, 2011, 533 outbreaks in neonatal patients had been recorded. Of these outbreaks, 68 (12.7%) were related to viruses. This database also reports that the most common pathogens in viral outbreaks were rotavirus (23.5%), RSV (16.2%), enterovirus (16.2%), adenoavirus (10.3%) and norovirus (8.5%). The most frequent type of presentation was gastrointestinal (including hepatitis) (55.9%) followed by lower respiratory tract infection (44.1%). A seasonal peak seemed to occur in winter in our study. Published data on the subject would indicate that there is little seasonal variation of pathogens recovered from NICU. Sporadic peaks of HPeV infections have been reported in young children.27,28

This study has a number of limitations. It is a small study and included 84 episodes of investigated sepsis. There were few term infants in the study, which may have altered the incidence. Picornaviruses can readily be detected in feces but these were not analyzed in this study. Viral quantification may also have been useful to determine the degree of viremia. These aspects could be combined in a larger viral-focused study to determine the true prevalence and types of viral disease in preterm infants and the relationship with concurrent bacterial infection. The rates of blood culture positivity were similar between the viral positive and negative groups. This may reflect that the infants were coinfected with 2 organisms or a high rate of blood culture contamination. The rate of sepsis (as defined by the NEO-KISS score) was higher in the viral positive group but this did not reach statistical significance. All infants included in the study did so based on the symptomatic presentation. The NEO-KISS score is a system for blood stream infection surveillance divided into 3 sections; clinical sepsis (without pathogen in blood culture) and laboratory confirmed blood stream infection with proof of pathogen (CNS or non-CNS). To be considered “sepsis positive”, a clinical and laboratory criteria threshold need to be met.29 Infants were assessed using these criteria to give an independent determination as to the presence of sepsis. The NEO-KISS score is used to determine sepsis of bacterial origin. Its performance in infants with viral infection is unpredictable. In our study, 5 of 11 HPeV positive infants were scored as sepsis positive, 6 of 11 were scored as sepsis negative. Although we excluded infants with NEC further study of viral incidence specifically in such infants may be of important.

Viral infections require additional vigilance and specifically targeted investigations. It is apparent that due to the variable nature of the presentation of preterm infections, viral origin of disease needs to be considered in all cases of possible infection despite the low recovery of pathogens. Ongoing surveillance is required to determine the true incidence of this infection and to determine whether it is a significant burden of disease.11,30

**REFERENCES**


