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Short communication

HPeV-3 predominated among *Parechovirus A* positive infants during an outbreak in 2013–2014 in Queensland, Australia

Donna McNeale[a], Claire Y.T. Wang[a,b], Katherine E. Arden[a], Ian M. Mackay[a,*]

[a] Child Health Research Centre, The University of Queensland, South Brisbane, Queensland 4101, Australia
[b] Centre for Children's Health Research, Children's Health Queensland, South Brisbane, Queensland, 4101, Australia

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ABSTRACT

Background: Parechoviruses (HPeV) are endemic seasonal pathogens detected from the respiratory tract, gut, blood and central nervous system (CNS) of children and adults, sometimes in conjunction with a range of acute illnesses. HPeV CNS infection may lead to neurodevelopmental sequelae, especially following infection by HPeV-3, hence screening and genotyping are important to inform epidemiology, aetiology and prognosis.
Objectives: To identify and characterise HPeVs circulating during an outbreak between November 2013 and April 2014 in Queensland, Australia.
Study design: To perform PCR-based screening and comparative nucleotide sequence analysis on samples from children with clinically suspected infections submitted to a research laboratory for HPeV investigations.
Results: HPeVs were detected among 25/62 samples, identified as HPeV-3 from 23 that could be genotyped. These variants closely matched those which have occurred worldwide and in other States of Australia.
Conclusions: The inclusion of PCR-based HPeV testing is not systematically applied but should be considered essential for children under 3 months of age with CNS symptoms as should long-term follow-up of severe sepsis-like cases.

1. Background

The species *Parechovirus A* include human parechovirus (HPeV) type 1 and 2, first identified in 1956 as echovirus 22 and 23 [1,2]. There are now 19 HPeV genotypes within the genus *Parechovirus*, family *Picornaviridae* [1]. Infection has been associated with respiratory [3,4] and gastrointestinal disease [4,5]. Young children are more likely than adults to suffer aseptic meningitis [4], encephalitis, flaccid paralysis and severe neonatal sepsis [4,6–10]. Long term neurodevelopmental sequelae from HPeV infection have also been suggested [11]. HPeV infections usually peak in warmer months with HPeV-3 the most frequently identified genotypes in central nervous system (CNS)-related infections, sometimes with rash and seizure [8,12–15]. Widespread infection is supported by seroprevalence studies noting > 85% of adults have pre-existing antibodies to HPeV-1 or 3 [16,17]. Recent findings indicate antigenic variation exists within the HPeV-3 [18]. Transmission is thought to be by droplets and after contact with contaminated surfaces [19,20].

In clinical practice, a primary focus is rapid identification of treatable causes of CNS infection but this may mean untreatable viruses like HPeVs are not strategically or routinely sought, perhaps comprising some of the many laboratory negative CNS samples [10,21–23]. Detection and genotyping of HPeVs in CSF samples supports the diagnosis of paediatric patients with acute CNS disease, reduces length of hospital stay and is best achieved using reverse transcriptase real-time polymerase chain reaction (RT-rtPCR) [10,24,25].

2. Objectives

During 2013–2014, reports described HPeV-3 cases in young children with acute CNS disease in the Australian east coast state of New South Wales (NSW) and southern state of Victoria [15,26–29]. These reports led us to offer research-based PCR testing to seek out and characterise the genotype of HPeV clinically suspected of causing similar cases in Queensland. We summarize this molecular epidemiology investigation.

3. Study design

3.1. Specimens

A range of samples were sent to the laboratory. Patients included

* Corresponding author. Present address: Public Health Virology, FSS, 39 Kessels Road, Coopers Plains Qld 4108, Australia.
E-mail address: ian.mackay@uq.edu.au (I.M. Mackay).

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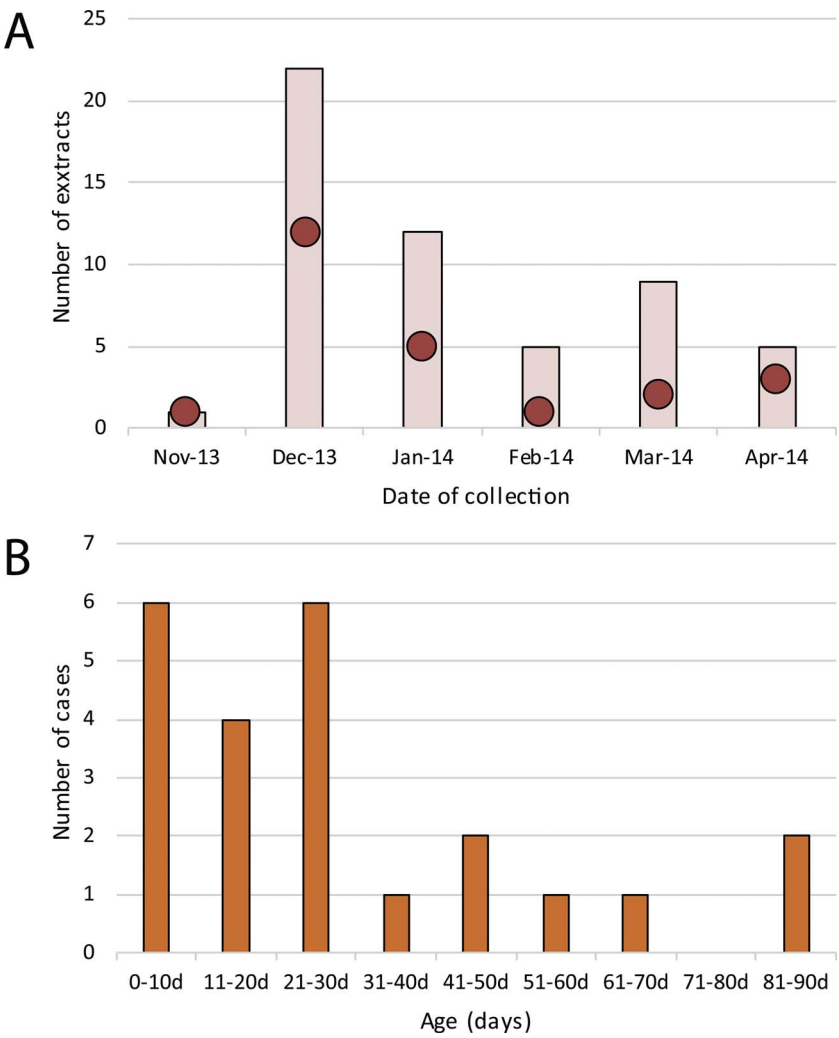

Fig. 1. Epidemiology of HPeV detections. A. The total number (bar), and number of HPeV positive samples (circle), by month studied, 2013 and 2014. B. Age distribution of the HPeV-positive cases. https://doi.org/10.6084/m9.figshare.5625055 .v1.

those with suspected encephalitis because of fever, seizures and abnormal findings in CSF (e.g. pleocytosis) or upon neuroimaging [11]. Unlinked sex, date of birth and date of specimen collection data were examined. In response to a clinical request, RNA extracts were provided by Pathology Queensland Central laboratory and were screened for *Parechovirus A* at our research laboratory. RNA had been extracted from 200 µl of sample using the Total Nucleic Acid Kit in the MagNAPure (Roche, Germany; eluted in 100 µl).

3.2. Parechovirus A detection

Extracts were screened using a previously described real-time RT-PCR targeting the 5′UTR. (Supp Table 1) [30] The RT-rtPCR employed 100 nM of each oligonucleotide in a 20 µl reaction mix (SensiFAST OneStep Mix; Bioline, Australia) including RNase inhibitor and 5 mM $MgCl_2$. After 2 µl of nucleic acid extract was added and reverse transcribed for 20 min at 45 °C, mixes were incubated at 94 °C for 2 min then cycled through 45 rounds of 94 °C for 15 s, 58 °C for 30 s and 72 °C for 10 s on a RotorGene 6000.

3.3. Parechovirus A genotyping

Genotyped employed a conventional nested RT-PCR assay that amplified 256nt encompassing part of the viral capsid protein coding regions VP3 and VP1 between nucleotide 2182 and 2437 (numbering excludes primers and is based on prototype sequence L02971; Supp Table 1) [25]. In our experience, this region is more likely to be successfully amplified than the VP1 region. The RT-PCR included 600 nM external oligonucleotides in a 20 µl reaction mix (SensiFAST OneStep Mix, Bioline, Australia) with RNase inhibitor and 3 mM $MgCl_2$. Two microliters of RT-PCR product were added to 18 µl PCR reaction mixes (MyTaq HS DNA Polymerase; Bioline, Australia) containing 380 nM internal oligonucleotides and 4.75 mM $MgCl_2$. Mixes were incubated at 94 °C for 1 min then cycled through 40 rounds of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 105 s followed by a 7 min incubation at 72 °C. Amplicons were electrophoresed to confirm presence and size of amplicon then sequenced (BigDye sequencing kit v3.1, Applied Biosystems Pty. Ltd; Australian Equine Genetics Research Centre, The University of Queensland). Following *in silico* removal of the primer sequence (Geneious Pro v8.1)[14], *Parechovirus A* sequences were assigned GenBank accession numbers KX579839-KX579861.

Sequences were compared to the GenBank database by BLASTn analysis and to other *Parechovirus A* sequences after multiple alignment and phylogenetic analysis. A sequence was assigned an HPeV genotype after meeting two criteria. Firstly, the new sequence had to cluster with a picornavirus study group (PSG)-assigned genotype in a phylogenetic analysis. Secondly, the sequence had to share 90% or greater nucleotide sequence identity with the relevant region of a fully sequenced PSG-characterised genotype. Analysis for recombination was not possible with this assay.

3.4. Approvals

This work was completed under the approvals issued by Queensland