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Characterising the molecular epidemiology of human parechovirus in young infants in the UK and Canada

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ABSTRACT

Objectives: We evaluated the extent of virus heterogeneity in PeV infected infants in the UK, Canada and Australia.
Methods: Samples were collected from PeV infected infants during 2013–16. Next generation sequencing was used to obtain sequencing data and construct phylogenetic trees based on analysis of the VP1 region. Comparison was made with sequencing data available from an outbreak in Australia.
Results: We amplified and sequenced 58 samples. All obtained PeV sequences were genotype 3 apart from one UK sample which was PeV-A5. Phylogenetic analysis revealed that all strains clustered together on the same clade and showed no significant genetic variation. We saw no significant evidence of association between sequence and either clinical severity (defined by admission to paediatric intensive care), geographical origin (compared between Canada and U.K) or year of sample collection (samples sequenced during 2013 – 2018).
Conclusions: In this small cohort, sequencing data indicate that PeV circulating in the UK and Canada from 2013 to 18 are derived from a common ancestor. No association between disease severity and genetic sequence was seen in the UK or Canadian cohorts. Larger studies are required to support these findings.

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1. Introduction

Human parechoviruses (PeV) are small, non-enveloped RNA viruses that belong to the *Picornaviridae* family, genus *Parechovirus* assigned to species A. There are 19 known PeV types within this species, but the majority of significant disease is in infants under 3 months of age and is caused by type 3 (PeV-A3) [1,2]. PeV-A1, PeV-A4 and PeV-A6 are associated with less severe disease than PeV-A3 and most frequently cause mild gastroenteritis in children under two years of age [3].

In the UK, PeV-A3 is the second most common cause of viral meningitis in infants under 3 months [4]. A British Paediatric Surveillance Unit (BPSU) study showed that almost a quarter of infants with PeV-A3 meningitis required paediatric intensive care (17 %, 6/35) or high dependency care (6 %, 2/35) [5].

During 2017 – 18, the largest recorded global PeV epidemic occurred in south east Australia and PeV-A3 accounted for all cases of meningitis [6]. The majority (86 %; 335/388) occurred in infants less than 12 months old. Of those hospitalised, a fifth (18.3 %; 34/186) required admission to intensive care.

In this study, the molecular epidemiology of PeV strains infecting infants from the UK and Canada was characterised using Next Generation Sequencing in the VP1 region and the sequences obtained were compared to those associated with the outbreak in east Australia during 2017–18. Our primary objective was to investigate the genetic relationship and potential spread of parechoviruses associated with outbreaks of severe disease in Australia with those subsequently circulating in the UK and Canada.

2. Materials and methods

2.1. Population

The Childhood Meningitis and Encephalitis (ChIMES) study recruited in 20 National Health Service (NHS) hospitals across the U.K during 2013 – 2016 [4]. Any child less than the age of 16 years old who had a lumbar puncture taken as part of a sepsis screen was eligible for enrolment. In cases recruited with PeV disease, thirteen CSF samples were obtained and submitted for NGS.

Public Health England encourages local NHS laboratories to submit PeV samples in order to conduct typing as part of ongoing disease surveillance. PHE submitted 31 (14 CSF, 6 stool, 5 upper respiratory and 4 serum) samples for NGS. All samples were collected in 2018.

In Vancouver, Brisitsh Colombia, CSF samples collected between 2013 and 2016 were tested for HPeV and identified 14 positive samples submitted to this study.

In the 2017 - 18 south east Australia PeV epidemic, 33 CSF samples were collected from hospitalised cases. Phylogenetic analysis based on partial 5′ and 3′ UTR from the Australian recombinant type 3 have been published and used in this study for comparison [7].

2.2. Data collection

Demographic (age and sex) clinical (admission to Paediatric Intensive Care Unit), laboratory (white cell count and CRP) and radiological (summary reports of neuroimaging) data for samples in the ChIMES study and from British Colombia were obtained (Table 1).

2.3. Next generation sequencing platform

Samples from the ChiMES study were sequenced using a metagenomic sequencing approach with enrichment as previously described [8] Viral sequencing of other samples was performed by NGS on an Illumina MiSeq platform using a metagenomic approach as previously reported [9]. Briefly, RNA was extracted from clinical samples using the NucliSENS magnetic extraction system (bioMerieux) and quantified using Qubit. As expected, most samples were CSF and had very low RNA concentrations which were not enough for RNA integrity number assessment using a Bioanalyzer. Metagenomic libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs) and 5 μl RNA / sample. After controlled fragmentation at 94 °C, RNA was reverse-transcribed and amplified (5–18 PCR cycles) using indexed primers. Libraries were quantified using Qubit, assessed for quality and size by TapeStation before normalisation and pooling forsequencing on a MiSeq machine.

2.4. Bioinformatic analysis

For bioinformatics analysis, low-quality bases were trimmed from

Table 1
Overview of markers of biochemical and clinical severity of cases with HPeV disease from the ChIMES and British Colombia cohorts.

Study cohort	Age (months)	sex	WCC (x10/9 L)	CRP (mg/L)	Admission to PICU	Neuroimaging
ChiMES	2	M	2.8	9.2	N	N
ChIMES	3	M	9.1	51	N	CT head (normal)
ChIMES	1	F	7.8	7	N	N
ChIMES	1	F	2.1	N/a	N	N
ChIMES	1	F	6	16.3	N	N
ChIMES	1	F	4.4	23	N	N
ChIMES	1	M	10.7	N/a	N	N
ChIMES	2	F	5.2	6	N	N
ChIMES	1	F	9.3	15	N	N
ChIMES	0	M	6.4	9.6	N	N
ChIMES	3	F	4.1	26	N	N
ChIMES	2	F	4.6	17.2	N	N
ChIMES	0	F	5.9	N/a	N	N
B.C	0	F	10	6	Y	CT head (normal)
B.C	0	M	11.3	9	N	N
B.C	1	M	5.3	13	N	N
B.C	1	M	3.9	12	N	N
B.C	0	M	5.5	2.5	N	N
B.C	1	M	4.8	20	N	N
B.C	1	M	5	N	N	N
B.C	0	M	8.9	7	N	N
B.C	0	M	6.4	<5	N	N
B.C	0	F	7.1	<5	N	N
B.C	1	M	3.7	13	N	N
B.C	0	F	4.3	<5	N	N
B.C	0	F	8	<5	N	N

de-multiplexed sequences using QUASR v7.01 (www.bioconductor.org/packages/release/bioc/html/QuasR.html) and adapter sequences removed using CutAdapt v1.7.1 (http://cutadapt.readthedocs.io/en/stable/index.html). Trimmed reads were mapped against reference sequences representing HPeV types using BWA-MEM [10] PCR primers were designed to fill sequence gaps when needed.

PeV-A3 sequences obtained from the study subjects were aligned to an assembled database of all available PeV-A3 VP1 sequences downloaded from GenBank in November 2019 (n = 694). Phylogenetic analysis was performed on sequences spanning the VP1 gene (positions 2336–3038 numbered using the PeV-A1 reference sequence L02971) using neighbour-joining of maximum composite likelihood corrected distances. Data was bootstrap resampled 100 times to estimate robustness of groupings; values of 70 % or greater are shown on supported branches.

3. Results

In total, 13 subjects (median age 39 days) from the ChIMES study, 31 (median age 2 months) from PHE and 13 (median age 29 days) from British Colombia had samples submitted for NGS. On average, each sample had more than 700,000 paired-end reads after quality trimming with the number of HPeV reads varing substantially between samples (Table 2). PeV genotype 3 was identified in 56 out of 57 samples. One sample obtained from respiratory secretions in a 13 month old child was identified as genotype 5. The median WCC (6.0 per mm^3 and 5.7 per mm^3) and CRP (15.5 mg/dL and 6.4 mg/dL) in the ChiMES and British Colombia cohorts were similar. One patient from the PHE and British Colombia groups were admitted to PICU (Table 1). We showed no association between severity of disease and sequencing data obtained.

Most genotype 3 sequences obtained from the PHE, ChiMES and Vancouver cohorts segregated into the same clade of VP1 region sequences (Fig 1). This clade also included PeV strains from the severely affected subjects in Australia in 2017–18 (yellow dots). Variants from ChiMES and Vancouver cohorts were largely distinct genetically, potentially reflecting their geographic or temporal separation. Both were however distinct from PeV-A3 strains from Australia, that clustered into separate sub-groupings that largely excluded the UK and Canadian origin variants. Within ChiMES and Vancouver cohorts, there was no specific clustering of variants based on their clinical severity although numbers compared are too small to draw any general conclusions.

Table 2
Overview of representative sequenced samples.

Sample ID	Cycle threshold	RNA concentration (ng/μl)	Total paired-end reads after trimming	Number of HPeV reads
S1013	20.94	8.4	531,311	141
S1050	35.38	<0.2	595,570	14
U1111	31.37	<0.2	979,358	222
U1113	29.28	<0.2	523,709	1452
U1207	39.3	<0.2	549,425	7
U1288	35.9	<0.2	523,881	16
U1299	34.56	<0.2	757,308	8
U1311	36	<0.2	712,915	26
U1319	35.28	<0.2	792,133	52
U1330	33.03	<0.2	510,645	95
U1408	33.15	<0.2	712,398	112
U1519	24.53	<0.2	784,273	27,336
PHE3	28	<0.2	1179,581	444
PHE6	32	<0.2	64,217	568
PHE9	28	6	1176,793	12
PHE17	32	<0.2	621,252	527
PHE18	27	<0.2	59,419	354
PHE21	28.5	<0.2	449,797	219
PHE22	27	<0.2	1246,946	50
PHE23	30	<0.2	2232,146	12
PHE26	24	<0.2	1799,766	298
PHE29	30	<0.2	729,847	33

4. Discussion

These data suggest that PeV samples obtained from this study across two continents are derived from a recent common ancestor. A surveillance study of PeV in Denmark during 2009 – 2012 and Holland during 2000 – 2007 also revealed little genetic variation [11,12]. This could be due to most infection being asymptomatic and therefore not evolving to escape the hosts immune response.

In our study, PeV-A3 was the by far the most common genotype identified and responsible for causing severe disease in several cohort members (Table 1), consistent with other cohort studies in the U.K, USA and Japan [13–15]. The high frequency of PeV3 samples does however reflect an acquisition bias as samples were largely derived from cases with suspected meningitis and therefore do not reflect the overall frequency of disease development associated with parechovirus infections. This small study provided no evidence that PeV-A3 infections from the UK or Canada (which were associated with moderate or severe disease) were any more closely related than variants characterised in the outbreak in Australia in 2017–2018 (that were predominantly caused by severe infection).

To our knowledge, we report the first known case of PeV5 circulating in the U.K. Cases of PeV5 have been reported previously in Australia, China, Denmark and the Netherlands [11,16]. Recombinant PeV5 has recently been described in south eastern Australia and shown to cause a sepsis-like illness in young infants [17].

This study has several limitations. Our cohort included samples collected through different protocols focusing on severe infections (ChIMES actively recruited cases of suspected meningitis whereas the samples collected by PHE and in Canada were not selected on the basis of clinical syndrome) and so there is marked heterogeneity in the cases identified and samples used. This is a small study which included only 14 samples from British Colombia. Clinical data were lacking for samples from PHE.

Currently, there are no phase II/III antiviral or vaccines trials against PeV [18]. In part, this is due to a lack of any robust molecular epidemiological data to inform interventional trials. Population based surveillance studies, which include sentinel sites and rely on syndromic testing, could develop the platform to collect these data [19]. The long term neurological outcomes of infants with severe PeV disease are poorly understood [20]. Future studies should characterise the molecular epidemiology of PeV disease in young infants in order to potentially inform future treatmtent trials and also better understand any role of host succeptibility factors in developing severe disease.

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CRediT authorship contribution statement

Seilesh Kadambari: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Resources, Writing – original draft, Writing – review & editing, Funding acquisition. **Heli Harvala:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dung Nguyen:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Manish Sadarangani:** Writing – review & editing, Methodology. **Natalie G Martin:** Resources, Writing – review & editing. **Ghada N. Al-Rawahi:** Resources, Writing – review & editing. **Inna Sekirov:** Writing

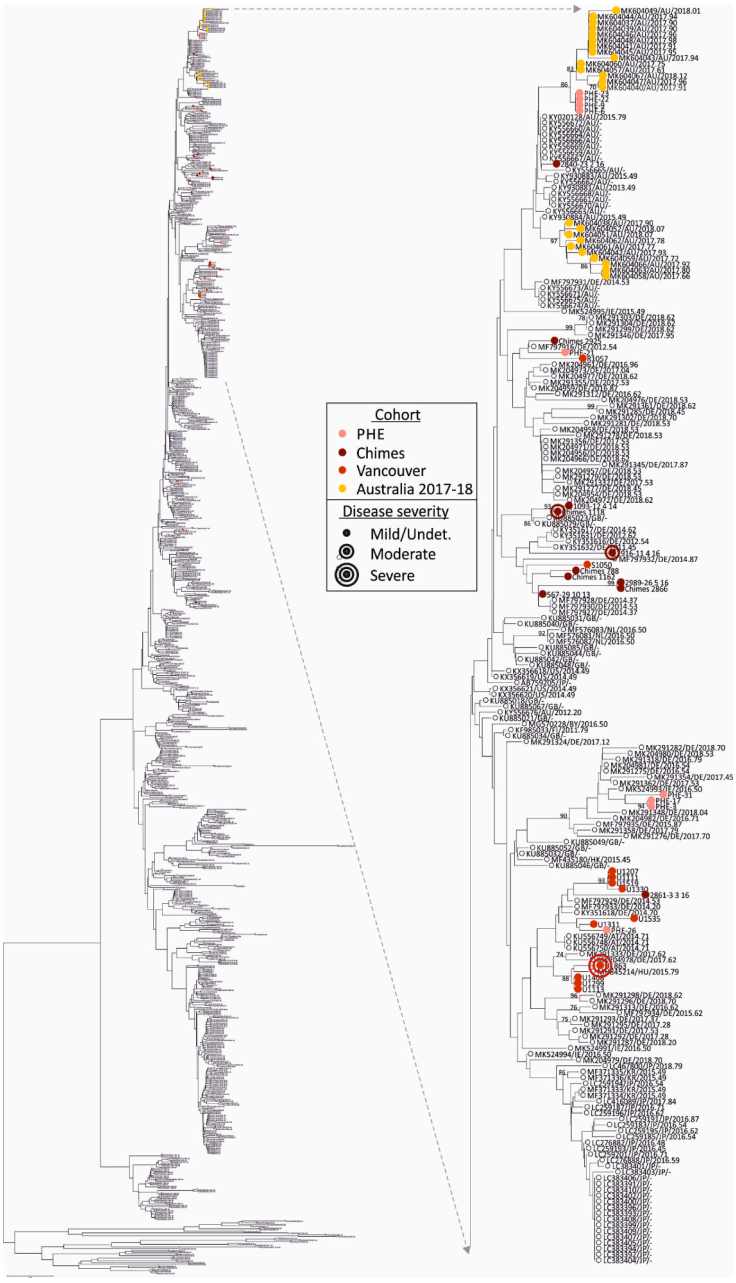

Fig. 1. Phylogenetic analysis of VP1 Sequences from study subjects and previously published genotype 3 parechovirus variants. The tree was built using neighbour-joining of maximum composite likelihood corrected distances. Bootstrap support for branches was obtained from 100 re-samples; values of 70 % or greater are shown.

– review & editing. **Sylviane Defres:** Writing – review & editing. **Tom Solomon:** Writing – review & editing. **Tanya Golubchik:** Methodology, Resources, Writing – review & editing. **Rory Bowden:** Methodology, Resources. **Andrew J Pollard:** Methodology, Resources, Writing – review & editing. **Peter Simmonds:** Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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