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VIROLOGY

Emergence of Parechovirus A4 Central Nervous System Infections among Infants in Kansas City, Missouri, USA

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ABSTRACT Among known parechovirus (PeV) types infecting humans, PeV-A3 (formerly HPeV3) and PeV-A1 (formerly HPeV1) are associated with pediatric central nervous system (CNS) infections. The prevalence of PeV-A3 among hospitalized infants with sepsis-like illness and viral CNS infection is well described; however, the contribution of PeV-A4 to infant CNS infection is relatively unexplored. We report the first 11 U.S. cases of PeV-A4 CNS infections occurring in Kansas City infants during 2010 to 2016 and compare the clinical presentation with that of PeV-A3. PeV-positive cerebrospinal fluid (CSF) specimens from 2010 to 2016 underwent sequencing for genotyping. Among all PeV-CSF positives, PeV-A4 was detected in 11 CSF samples from 2010 to 2016. PeV-A4 was first detected in 2010 ($n = 1/4$), followed by detections in 2014 ($n = 1/39$), 2015 ($n = 6/9$), and 2016 ($n = 3/33$). The median age of PeV-A4-infected infants in weeks (median, 4; range, 1 to 8) was similar to that of infants infected with PeV-A3 (median, 4; range, 0.25 to 8). Clinical characteristics of PeV-A4 ($n = 11$) were compared with those of select PeV-A3-infected children ($n = 34$) with CNS infections and found to be mostly similar, although maximum temperature was higher ($P = 0.017$) and fever duration was shorter ($P = 0.03$) for PeV-A4 than for PeV-A3. Laboratory test results were also similar between genotypes, although they showed significantly lower peripheral white blood cell ($P = 0.014$) and absolute lymphocyte ($P = 0.04$) counts for PeV-A4 infants. Like PeV-A3, PeV-A4 caused summer-fall seasonal clusters of CNS infections in infants, with mostly similar presentations. Further surveillance is necessary to confirm potential differences in laboratory findings and in fever intensity/duration.

KEYWORDS central nervous system infections, children, parechovirus

Human parechoviruses (PeV), originally discovered during a summer diarrhea outbreak in 1956 as echoviruses 22 and 23, were later reclassified in 1999 as a separate genus, *Parechovirus*. On the basis of genetic and biological differences (1, 2), the original two species were renamed PeV-A1 and PeV-A2. Parechoviruses belong to the largest RNA virus family, *Picornaviridae*, which includes other important and diverse groups of human pathogens known to cause aseptic meningitis, hand, foot, and mouth diseases, hepatitis, etc. (3) Members of this family are small (~30 nm), nonenveloped, positive-sense, single-stranded RNA viruses (4, 5) that are the etiological agents of respiratory, gastrointestinal, and central nervous system (CNS) infections (6, 7).

The genus *Parechovirus* is comprised of two species: parechovirus A and B. The former consists of 19 types (PeV-A1 to PeV-A19) (http://www.picornaviridae.com/parechovirus/parechovirus_a/parechovirus_a.htm) primarily associated with human infections, of which type 1 (PeV-A1) and type 3 (PeV-A3) are among the most frequently reported to cause infections worldwide (1, 4, 8). Parechovirus B, formally known as Ljungan virus, is pathogenic for animals (9). Symptomatic human PeV illness is most notable in neonates and infants and is expressed in a variety of presentations, ranging from mild gastrointestinal and respiratory infections to severe CNS infections and/or

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neonatal sepsis syndrome (10). PeV-A1 causes primarily asymptomatic to mildly symptomatic infections, while PeV-A3 is a major cause of sepsis-like illness and CNS infection in infants (9).

The fourth human parechovirus type (PeV-A4) was identified by virus neutralization assays in 2006 (11) in a stool sample of a 6-day-old infant with fever and feeding problems (12, 13). PeV-A4 is primarily associated with relatively mild infections of the gastrointestinal and respiratory tracts (13). Three cases of PeV-A4 CNS infections, one in France (14) and two in Finland (15), were described in 2010 and 2012, respectively. We describe PeV-A4 CNS infection presenting as sepsis-like illness in young infants over several years in the Kansas City area. We report the first U.S. PeV-A4 CNS infection, detected in 2010, and also describe the largest series of PeV-A4 CNS infections recorded worldwide.

MATERIALS AND METHODS

Clinical specimens. Between June 2010 and December 2016, a total of 3,093 pediatric cerebrospinal fluid (CSF) samples ($n = 389$ in 2010, $n = 330$ in 2011, $n = 462$ in 2012, $n = 433$ in 2013, $n = 519$ in 2014, $n = 480$ in 2015, and $n = 480$ in 2016) were submitted as part of standard-of-care management at the Children's Mercy Hospital, Kansas City (CMH-KC). The study was approved by the Institutional Review Board at CMH-KC.

Nucleic acid extraction and RT-PCR. For routine PeV detection, RNA extraction was carried out using the NUCLISENS easyMAG automated nucleic acid system (bioMérieux, Durham, NC) from an aliquot of a 200-μl CSF specimen and eluted in 55 μl of Tris-EDTA buffer. An internal control, MS2, was spiked in the CSF samples before extraction to monitor the efficiency of extraction and amplification. The extracted RNA was screened for PeV by a two-step pan-PeV real-time reverse transcription-PCR (RT-PCR) on an AB7500 fast real-time PCR system (Applied Biosystems, Foster City, CA), as previously described (16). The PeV-positive specimens were then subjected to genotyping in the CMH-KC genomics center.

Genotyping and sequence analysis. Genotyping of PeV-positive samples was carried out by reverse transcribing the extracted RNA using Superscript II (Invitrogen), followed by a two-step PeV-specific seminested RT-PCR assay to amplify the targeted VP1 region, as previously described (17, 18). Amplified products were run on a 1.8% agarose gel to observe bands corresponding to 500 to 600 bp in size. Appropriately sized amplicons were purified with either the ExoSAP PCR product cleanup reagent (Applied Biosystems) or the QIAquick gel extraction kit (Qiagen), and bidirectional sequencing was performed using the seminested forward and external reverse primers (18) with an AB Prism BigDye Terminator cycle sequencing kit, V.3.1, on an AB 3500XL DNA Sequencer (Applied Biosystems). Lasergene 14 software (DNASTAR) was used for sequence assembly and editing, followed by BLAST analysis to identify the PeV genotypes. A phylogenetic tree was constructed using the DNASTAR-MegaAlign software to see if all PeV-A4 isolates genotyped during the study period compared well with existing reference strains.

Clinical data. Clinical characteristics of all PeV-A4-infected children ($n = 11$) were compared with select PeV-A3-infected children ($n = 34$) with CNS infections from outbreaks during 2015 and 2016. Electronic medical records of PeV-positive children were reviewed to document the following: patient age and gender, length of hospital stay, medical history, presence and duration of fever (temperature of >38.5°C), maximum temperature (T_{max}), manifestations of sepsis-like illness, neurological symptoms, seizures, irritability, clinical diagnoses of meningitis or encephalitis, respiratory or gastrointestinal signs/symptoms, and rashes. Laboratory values for CSF and peripheral blood cell counts, CSF protein and glucose concentrations, bilirubin, liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), and C-reactive protein (CRP) were also documented when available. Any available magnetic resonance imaging, ultrasound, or chest radiograph results were also reviewed. In addition, use of antimicrobials during hospitalization and diagnoses at discharge were recorded (1).

Dichotomous values were analyzed by the Fisher exact test. Continuous values were analyzed by unpaired *t* test or Mann-Whitney testing as appropriate using Graphpad 3.10 or SigmaPlot 12.0.

RESULTS

PeV type identification. Routine PCR screening of 3,093 CSF samples from 2010 to 2016 detected PeV-A in 157 (5%) patients. Among the 127 successfully genotyped specimens, PeV-A3 accounted for 91% ($n = 116$) and PeV-A4 accounted for 9% ($n = 11$) (Fig. 1). We detected the first known PeV-A4 CNS infection in the United States in a Kansas City infant in 2010. Our second detection was in 2014. We detected the first U.S. outbreak of PeV-A4 CNS infections in infants ($n = 6$) in Kansas City during 2015 and a continued presence in 2016 ($n = 3$).

In the outbreak year (2015), 12/480 (3%) CSF specimens tested positive for PeV by real-time RT-PCR. The median threshold cycle (C_T) value for all PeV-positive samples in real-time RT-PCR was 35.46 (25.49 to 38.01). Of all 2015 PeV-positive samples, genotyping was successful for 9/12 (75%) specimens. There was insufficient nucleic acid

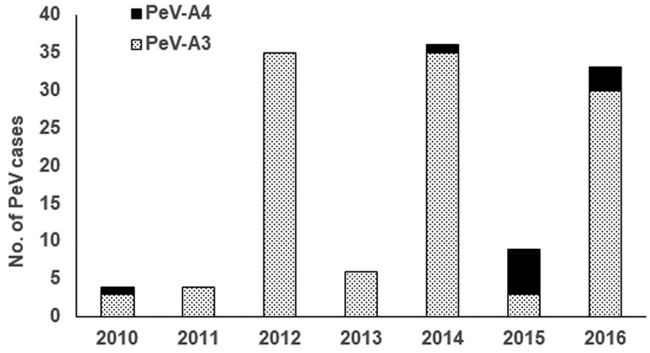

FIG 1 Detection frequencies of individual PeV genotypes over the study period, January 2010 to December 2016.

content for sequencing in three samples after initial PeV PCR. Virus typing confirmed that 6/9 sequenced samples were PeV-A4 (67%) and 3/9 were PeV-A3 (33%) (Fig. 1).

In 2016, 37/480 (8%) CSF specimens tested positive for PeV. Genotyping confirmed that 3/36 (8%) specimens were PeV-A4 and 31/36 (6%) were PeV-A3, and the remaining 3 failed sequencing. The median C_T value for all PeV-positive samples was 33.38 (range, 20.85 to 38.01). Overall median C_T values for PeV-A3 did not differ from those of PeV-A4 (PeV-A3 median, 33.4; range, 20.85 to 37.743; PeV-A4 median, 35.0; range, 30.38 to 38.01), with a *P* value of 0.887. Phylogenetic analysis confirmed the identities of all PeV isolates. All 9 of the PeV-A4 isolates formed a separate cluster, which included 3 reference strains from the Netherlands (DQ315670), France (JQ229466.1), and California (AM235750). The 34 PeV-A3 isolates were related to the reference strain AB084913 from Japan (Fig. 2).

PeV seasonal prevalence. Figure 3 shows the seasonal prevalence of PeV. Detection of both PeV-A3 and PeV-A4 peaked during the summer and fall seasons. The outbreak of PeV-A4 in 2015 occurred during August to December. PeV-A3 infections were detected somewhat earlier and PeV4 infection somewhat later in the year, but differences were not significant.

Clinical characteristics of PeV-infected children. Most of the PeV-positive infants presented with sepsis-like illness and were less than 1 month old (mean age, 3.84 ± 1.55 weeks; range, 6 days to 8 weeks). Table 1 shows a comparison of the clinical features and presentation of the 11 PeV-A4 patients and 34 PeV-A3 patients detected during January 2015 to December 2016. The clinical characteristics for the single PeV-A4 patient from 2010 and 2014 are included in the comparison. Overall, males predominated (58% versus 42% females), and most were under 1 month of age, regardless of whether their infections were due to PeV-A3 or PeV-A4.

Laboratory value comparisons. Infants infected with PeV-A4 and PeV-A3 did not differ significantly in CSF laboratory findings. CSF pleocytosis was absent, and CSF white blood cell (WBC) counts were within the normal range for all PeV subjects (0 to 10 WBC/mm^3), with no significant difference between PeV-A3 and PeV-A4. Overall, 63% of PeV-infected infants had low glucose concentrations in their CSF, while 29% had elevated CSF protein concentrations compared to the normal reference range. Two of the PeV-infected infants with low glucose and elevated protein concentrations had visibly bloody CSF.

When PeV-A4 and PeV-A3 were compared, lower than normal CSF glucose concentrations were noted in 4/9 (44%) PeV-A4-infected and 22/34 (65%) PeV-A3-infected infants ($P = 0.999$), with a mean of 43.25 ± 2.58 mg/dl (range, 39 to 46 mg/dl) and 47.72 ± 2.70 mg/dl (range, 40 to 49 mg/dl), respectively. An elevated CSF protein concentration was seen in 4/9 (44%) PeV-A4-infected infants (mean, 65 ± 2.73 mg/dl; range, 62 to 69 mg/dl) and in 8/34 (24%) PeV-A3-infected infants (mean, 78.12 ± 16.20 mg/dl; range, 56 to 98 mg/dl).

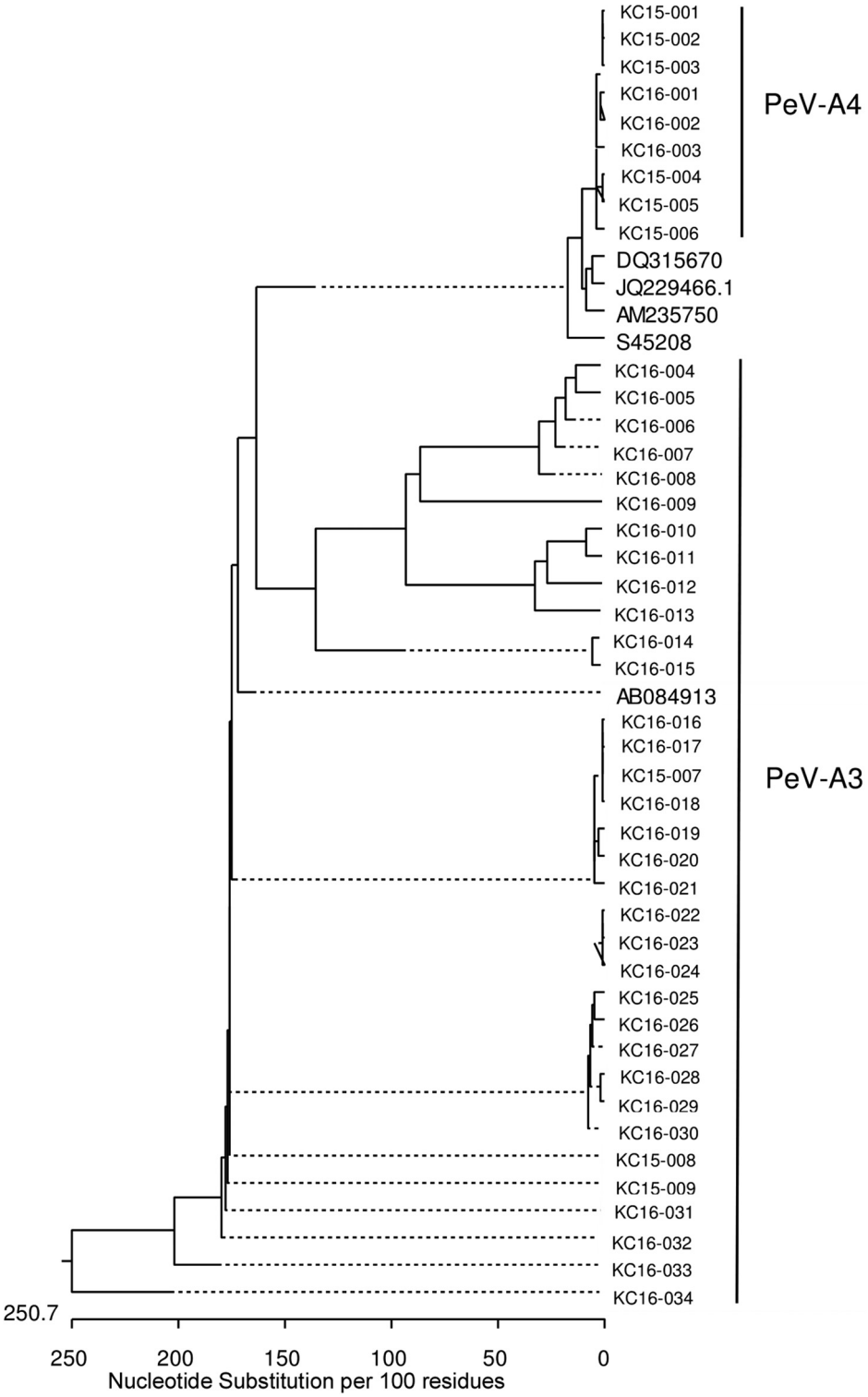

FIG 2 Phylogenetic comparison of complete VP1 coding sequences of PeV strains. The analysis included the 43 PeV sequences (34 PeV-A3, 9 PeV-A4). Reference strains from GenBank included three PeV-A4 (DQ315670, JQ229466, and AM235750), one PeV-A3 (AB084913), and one PeV-A1 (S45208) strain. The tree was constructed using the DNASTAR-MegaAlign software.

For all PeV patients, peripheral WBC counts were lower than normal in 21/43 (49%) patients, with a mean of 4.06 ± 0.63 mg/dl (range, 2.67 to 14.67 mg/dl), and were significantly different between PeV-A4 and PeV-A3 ($P = 0.0138$) (Table 1). Absolute lymphocyte count (ALC) values were also lower in PeV-A4 than in PeV-A3 patients

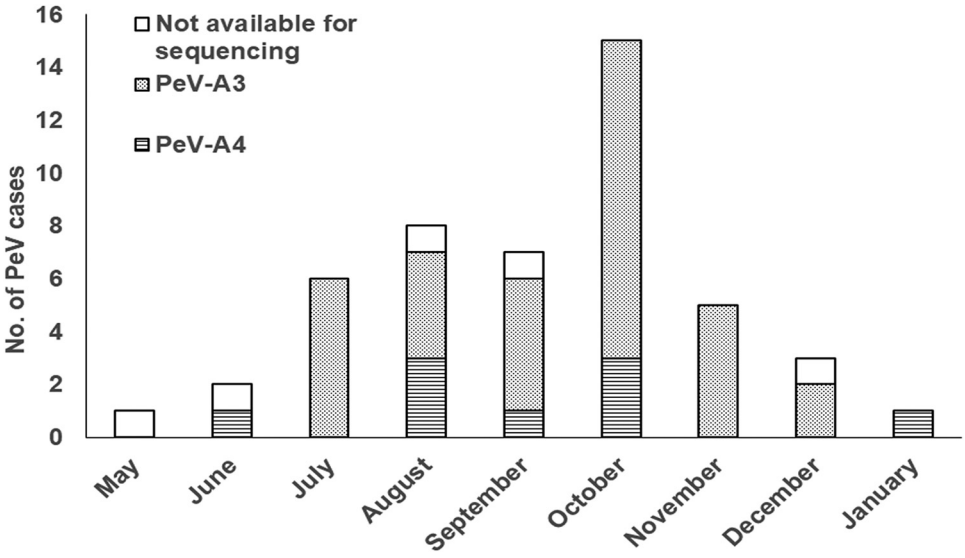

FIG 3 Seasonal incidence of PeV infections during the outbreak year, January 2015 to December 2016 ($n = 43$).

($P = 0.041$). Other laboratory result values, such as CRP, absolute neutrophil count (ANC), AST, and ALT, also were not significantly different between the PeV-A3 and PeV-A4 groups (Table 1).

Clinical course of PeV-infected children. The most commonly observed symptoms were fever, poor feeding, dehydration, irritability, rashes, tachycardia, tachypnea, and respiratory distress. The symptoms did not differ between groups. Children with PeV-A4 infection had significantly higher T_{max} during hospitalization, with a mean value of 39.2 ± 0.4°C (range, 38.7 to 40.1°C), than PeV-A3-infected patients (mean, 38.8 ± 0.4°C; range, 38 to 39.6°C) ($P = 0.017$). However, fever duration was longer in PeV-A3 than PeV-A4 infection (2.1 ± 0.96 versus 1.5 ± 0.52) ($P = 0.03$). The PeV-A4 group showed no difference in length of stay from that of PeV-A3-infected infants. All patients in each group were treated empirically with antibiotics and acyclovir before PeV testing results were known. Two (2/45) infants, one with each genotype, were considered ill enough to require intensive care. On the day of discharge, all infants were afebrile, feeding well, and in good condition, with no identified neurological signs or symptoms.

DISCUSSION

Of the 19 identified PeV genotypes worldwide, data about some genotypes remain limited (19). Recent studies have shown that at least 2 PeV types, PeV-A1 to some extent and, more frequently, PeV-A3, are associated with potentially severe sepsis-like illness and CNS infections, particularly in young infants (9, 13, 20, 21). In this study, we

TABLE 1 Comparison of clinical and laboratory features and presentations of PeV-A4 with PeV-A3

Clinical feature (reference range)	Value for [mean ± SD (range)]: PeV-A3 ($n = 34$ unless otherwise noted)	PeV-A4 ($n = 11$ unless otherwise noted)	*P* value
Hospitalization days[a]	3.09 ± 2.08 (0–9)	2.90 ± 0.88 (0–5)	0.256
Days of fever	2.1 ± 0.96[a] (1–4)	1.55 ± 0.52 (1–2)	0.029
T_{max} (>38°C)	38.82 ± 0.44[a] (34.7–40.3)	39.17 ± 0.39 (38.7–40.1)	0.017
Peripheral WBC (9×10^3 to 17.5×10^3 cells/μl)	6.59 ± 3.19 (2.67–13.37)	4.69 ± 1.49 (2.96–7.66)	0.0138
Absolute neutrophil count (1.5×10^3 to 8.5×10^3 cells/μl)	2.71 ± 2.28 (0.52–9.08)	2.45 ± 1.25 (0.92–4.2)	0.7337
Absolute lymphocyte count (4×10^3 to 10.5×10^3 cells/μl)	2.33 ± 1.51 (0.86–7.36)	1.42 ± 0.74 (0.62–3.05)	0.0626
CSF WBC (0-5 cells/mm^3) ($n = 33$ PeV-A3 and $n = 7$ PeV-A4)	2.88 ± 2.50 (0–10)	3.14 ± 2.61 (0–7)	0.8791
CSF glucose (50–80 mg/dl) ($n = 34$ PeV-A3 and $n = 8$ PeV-A4)	47.6 ± 5.43 (40–64)	47.75 ± 5.28 (39–54)	0.9999
CSF protein (12–72 mg/dl) ($n = 33$ PeV-A3 and $n = 7$ PeV-A4)	57.67 ± 18.82 (23–98)	58.43 ± 12.14 (41–69)	0.9988

[a]Three PeV-A3 patients were still febrile on day of discharge. Two PeV-A3 patients were hypothermic at presentation.

report the first detected PeV-A4 CNS infection in the United States in 2010 and a later modest outbreak of PeV-A4 CNS infections in young infants during 2015 to 2016. Two infants with PeV-A4 CNS infection were judged sufficiently ill to require treatment in the intensive care unit.

There have been prior PeV-A4 reports outside the United States associated with fever and mild infections of gastrointestinal and/or respiratory tracts in older children (mean age, 14 months) but no clear association with CNS-related disease (13). However, a 2010 French study identified PeV-A4 in the CSF of 1/1,128 patients presenting with neonatal fever (14). Recently, a 2012 Finland-based study reported PeV-A4 in the blood of 2 suspected sepsis cases (22). Moreover, the same group of researchers in 2012 identified PeV-A4 in the CSF of 2 patients presenting with neonatal fever (15). Nevertheless, PeV-A4 has rarely been detected in hospitalized children. We report that PeV-A4 accounted for 21% of PeV-positive CSF in a 2-year period (2015 to 2016) and 2 other sporadic cases in infants with sepsis-like illness. PeV-A4 was not detected among our patients in 3 other previous PeV outbreak years (2007, 2009, and 2012), where all detections were for PeV-A3. The seasonal prevalence of PeV-A3 and A4 infections was similar and seen during summer-fall seasons.

There were no differences in age or gender between PeV-A4 and PeV-A3 patients. Infections were most common in infants less than 1 month of age, with a higher percentage of males in both groups. The clinical features and symptoms presented by PeV-A4 CNS-infected infants resembled those of PeV-A3 CNS infection (23), with a few exceptions, including higher maximum temperature, shorter fever duration, and lower white blood cell counts for PeV-A4.

Maximum recorded temperature values were statistically higher in PeV-A4 than PeV-A3 patients; however, a 0.3°C difference may not be clinically useful given the considerable overlap of fever values between patients with the 2 PeV types. The utility of the half-day-longer fever in predicting PeV-A4 infection is also unclear. Lower peripheral WBC counts were mostly due to lower lymphocyte, not neutrophil, counts in PeV-A4 patients. We had insufficient C-reactive protein data to determine if there was any similar signal, with testing available in only 3 PeV-A3 patients (all normal) and 3 PeV-A4 patients (all abnormal). Taken together, the results from our modest-sized data set suggest a somewhat different response between the 2 genotypes. Future studies may or may not reveal a more brisk inflammatory response in PeV-A4 patients.

Thus, our findings demonstrate that PeV-A4 can cause CNS infection and present as sepsis-like illness in young infants. We have described the largest series of PeV-A4 CNS infection in infants to date worldwide. Our first case of PeV-A4 CNS infection detected was similar to that detected in the infant in France in 2010 (14). PeV-A4 was the predominant PeV type in 2015, accounting for 67% (6/9) of all PeV in that year; interestingly, major outbreaks of PeV CNS infections comprised primarily of PeV-A3 have occurred during even-numbered years for the last three (2012, 2014, and 2016) of the five detected PeV outbreaks in Kansas City up to 2016.

The two PeV-A4 cases identified in Finland were similar to our 11 PeV-A4 cases, e.g., a 2-week-old infant and a 6-week-old infant with high fever, ALC of 3.4×10^9/liter and 7.7×10^9/liter, and CSF protein of 42.3 and 48.8 mg/dl, respectively. We did not detect acute sequelae of the 11 PeV-A4 CNS infections, but longer-term follow-up and more data are necessary to determine the long-term effects of PeV-A4 and whether they differ from those of PeV-A3.

To date, the National Enterovirus Surveillance System (NESS) has reported PeV-A3 to be the predominant parechovirus type circulating in the United States (4, 24, 25), and PeV-A4 outbreaks have not been described in the United States before this study. More data on PeV genotypes are needed to further differentiate the PeV-A4 from PeV-A3 disease burden.

Since most infants with PeV-positive CSF have no pleocytosis (2, 9, 26, 27), our data underscore the need for clinicians to consider testing for PeV regardless of CSF leukocyte count values in order to optimize diagnosis and management of infants presenting with symptoms that necessitate a sepsis work-up. Differentiation of PeV types for clinical management may not be necessary, since no specific treatment or

differences in clinical course is noticed between the two PeV types; however, epidemiological studies should consider PeV typing to see if clinical course and outcomes vary between PeV types in future outbreaks. *In vitro* evaluation of innate immune responses and intracellular signaling comparing PeV-A4 to PeV-A3 CNS strains appears warranted. Prospective clinical studies will also be needed to further clarify the role of PeV-A4 in infant CNS infections, the long-term outcome of such infections, and the true prevalence over time (6, 9). Rapid detection and reporting of PeV from CSF specimens could reduce length of stay and antimicrobial utilization in hospitalized infants.

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