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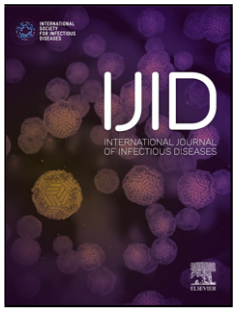


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# Human parechovirus type 5 neurological infection in neonate with favorable outcome: a case report

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## Highlights

- First Italian parechovirus type 5 neonatal infection
- PeV-A5 was detected and sequenced in both CSF and plasma samples
- Parechovirus should be searched in infants fever and/or sepsis like syndrome

**Abstract**

The majority of Parechovirus A (PeV) type 5 (PeV-A5) infections have been reported in patients with gastrointestinal syndromes. In contrast, sepsis-like illness associated with PeV-A5 infections have been reported only anecdoctically. Herein, we report the first case in Italy of PeV-A5 neurologic infection presenting in a neonate with sepsis-like syndrome. The patient, a healthy 41-weeks' gestation male was highly distressed and inconsolable, and had been persistently crying, with poor breastfeeding since the previous day. From day 2 to 4, the newborn was feverish with mild irritability; breastfeeding was preserved and regularly supported. His clinical condition progressively improved, with defervescence on day 4. He was discharged after seven days and neurological examination results indicated only mild impairment in visual fixation and vertical eye tracking and mild axial hypotonia. The Italian PeV-A5 strain was phylogenetically related to three strains detected in Denmark in 2012 as well as one detected in Australia and one in Greece in 2015 with an average nucleotide identity of 97.9% (range 95.9-100.0%). Enterovirus/PeV infection in newborns should be ruled out in cases of infants with unexplained fever and/or sepsis like syndrome and/or meningoencephalitis; etiological diagnosis is essential to avoid unnecessary administration of antibiotics and to plan long-term follow-up until schooling.

*Keywords:* neonatal infections, human parechovirus type 5, CSF, molecular characterization, CNS infection, phylogenetic analysis.

**Introduction**

Parechoviruses A (PeV-A) are an increasingly recognized cause of meningo-encephalitis in children and PeV-A type 3 (PeV-A3) is thought to be particularly neurotropic, as it is frequently identified in the cerebrospinal fluid (CSF) of infants with sepsis-like presentations [Wolthers et al., 2008; Harvala et al., 2010]. On the contrary, the large majority of PeV-A type 5 (PeV-A5) have been identified in stool samples of patients with gastrointestinal syndromes [Graul et al., 2017;

Pajkrt et al., 2009; van der Sanden et al., 2008; Zhang et al., 2011; Zhong et al., 2011]. Indeed, PeV-A5 has seldom been reported as a cause of meningo-encephalitis or sepsis-like syndromes [Cabrerizo et al., 2015]. Herein, we report the first Italian case of PeV-A5 neurological infection observed in a neonate with sepsis-like syndrome.

**Case report**

A healthy 41-weeks' gestation male born in November 2018 by vaginal delivery presented at 15 days old to the Emergency Room of F. Del Ponte Hospital in Varese. The newborn was highly distressed and inconsolable, and had been persistently crying, with poor breastfeeding and low-grade fever (37.6°C) since the previous day. There had been contact with an older sister suffering from otitis. He was admitted without any respiratory or gastrointestinal signs and symptoms; vital signs were stable with HR 130 ppm, RR 40 bpm and pulse oximetry 99%. As summarized in Table 1, laboratory tests on admission revealed a slight increase in CRP levels (13,2 mg/L), mild leucopenia with marked relative monocytosis (WBC 4550/$mm^3$, monocytes 24%, neutrophils 34%, lymphocytes 41%) and normal Hb and platelet count. Blood and urine samples were collected for culture and analysis and the baby was admitted at the neonatal intensive care unit (NICU). Soon after admission, he became whiny and developed a high fever (39°C), in addition to high heart rate (200 bpm at rest) with cutaneous vasoconstriction and cold extremities. Axial hypotonia was observed during a neurological examination and a lumbar puncture was therefore performed, revealing normal physical, chemical and microscopic CSF. Broad spectrum antibiotic therapy with ampicillin and amikacin was started, as a central nervous system (CNS) disease was suspected. Intravenous acyclovir was not administered due to negative real-time PCR for herpes simplex type 1 and 2 DNA in CSF. Rotavirus, adenovirus, norovirus, astrovirus, and sapovirus fecal antigens were negative. On day 1, a Cranial ultrasound performed on day 1was negative.

From day 2 to day 4, the newborn was feverish with mild irritability, breastfeeding was preserved and regularly supported. His clinical condition progressively improved, with

defervescence on day 4. Antimicrobials were discontinued after blood, urine and CSF tests came back negative.

On day 5, CSF and blood samples were sent to the Molecular Virology Unit of the Fondazione IRCCS Policlinico San Matteo, Pavia to investigate possible enterovirus and PeVs infections. CSF and plasma samples were positive in HPeV-specific real-time RT-PCR assays [Nix et al., 2008]. Genotyping was performed by sequencing amplicons obtained for both clinical samples using nested RT-PCR [Harvala et al., 2008]. Phylogenetic analysis of the sequences obtained indicated that the infection was sustained by a PeV-A5 strain. The newborn was discharged after seven days and neurological examination results indicated only mild impairment in visual fixation and vertical eye tracking and mild axial hypotonia. At 1 month from onset, the MRI scan was normal and and there was no evidence of white matter lesions. At the first follow-up appointment in January 2019, he demonstrated normal psychomotor development with a significant recovery of visual function.

**Phylogenetic analysis**

A total of 21 PeV-A5 sequences retrieved from the GenBank database matched the genomic region sequenced (partial VP3/P1). Overall, based on the maximum likelihood tree obtained, three different clades were identified (named as A-C in Figure 1). The average nucleotide identity between sequences belonging to the clade C was 87.5% (range 64.3%–100.0%). However, if we focused the analysis on strains that were closely related to the Italian strains (accession numbers MN067978-79) three of them were detected in Denmark in 2012, one in Australia and one in Greece in 2015). The average nucleotide identity increased to 97.9% (range 95.9-100.0%). Significantly, it is worth noting that the vast majority of sequences were obtained from PeV-A5 strains detected in stool samples. To the best of our knowledge, our PeV-A5 strain is the first to be sequenced directly from CSF.

**Discussion**

Signs of sepsis in neonates are often non-specific and a high level of alertness is needed for early diagnosis. The introduction of PeV screening in clinical diagnostic laboratories has improved the diagnosis of PeV infections, especially in clinical samples from patients with neonatal sepsis or neurological syndromes [Harvala and Simmonds 2009]. Among PeVs, PeV-A3 is known to induce neurological signs like hypotonia, irritability, drowsiness and seizures, and other generic signs and symptoms including fever, diarrhea, abdominal distension, apnea and rash [de Crom et al., 2016]. PeV infections have been reported in Italy since 2008 and the great majority of them were due to PeV-A3 [Piralla et al., 2012; Pariani et al., 2014; Piralla et al., 2014; Bubba et al., 2017]. There have been no reports of PeV-A5 circulating in Italy nor of PeV-A5 in patients with sepsis-like and neurological syndrome. PeV-A5 has been predominantly detected in the stools of patients with mild gastroenteritis, along with a few retrospective cases of PeV-A5 infections that were associated with more severe syndromes [Cabrerizo et al., 2015]. In our case, PeV-A5 was detected and sequenced in both CSF and plasma samples of patients with signs and symptoms of sepsis-like illness that resolved clinical syndromes in less than 7 days with a favorable outcome. Currently we cannot exclude the appearance of long-term neurodevelopmental sequelae but the full recovery of psychomotor development seems to be a good sign. Short-term outcomes are generally good, but medium and long- term outcomes of infants after PeV infections are still unknown; there are very few studies on the topic and those available are limited to small sample sizes with different methodology [Martin Del Valle et al., 2019; Britton et al., 2016; Verboon-Maciolek et al., 2008]. In general, the prognosis is more favorable when there is no CSF injury whereas infants with encephalitis (alterations in neuroimaging and/or seizures) are more likely to develop neurological disability and white matter injury disease [Britton et al., 2016]. PeV meningoencephalitis is often difficult to identify because clinical presentation of signs and symptoms of neurological involvement are non-specific in newborns and young infants. Simple detection of PeV in CSF is not predictive of CNS disease and should not be used as a negative prognosis marker [Harvala et al.,

2014]. Seizures, fever, apnea or prematurity appear to be risk factors for neurologic sequelae, but further studies are required to confirm this [Joseph et al., 2019].

In our case, the newborn was a full-term, healthy baby and his symptoms of irritability, fever and tachycardia were more related to sepsis-like syndrome; CNS involvement was mild, there were no seizures and intensive support was not needed. As previously observed in neonatal PeVs CNS infections, pleocytosis or biochemical alterations in CSF are usually absent [Black et al., 2019; Verboon-Maciolek et al., 2008]. A recent study of 29 cases of PeV neurological infections showed that in all cases CSF had less than 20 white cells per $mm^3$ [Kadambari et al., 2019]. In line with these observations, in our case all CSF parameters were normal and pleocytosis was absent. Finally, rapid testing using real-time RT-PCR could be considered the gold standard for PeV diagnosis, although antiviral drugs against these infections have yet to be approved and treatment is limited to supportive care. In addition, the use of multiple clinical samples such as CSF, respiratory, blood, urine, and stool specimens could improve detection rates of PeV infections. In conclusion, PeV infection in newborns should be ruled out in cases of infants with unexplained fever and/or with sepsis-like syndrome and/or meningoencephalitis; etiological diagnosis is essential to avoid unnecessary administration of antibiotics and to plan long-term follow-up until schooling.

**Conflict of interest**

The authors declare no conflict of interest

**Funding Source**

None.

**Ethical Approval**

Approval was not required.

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**Figure legend**

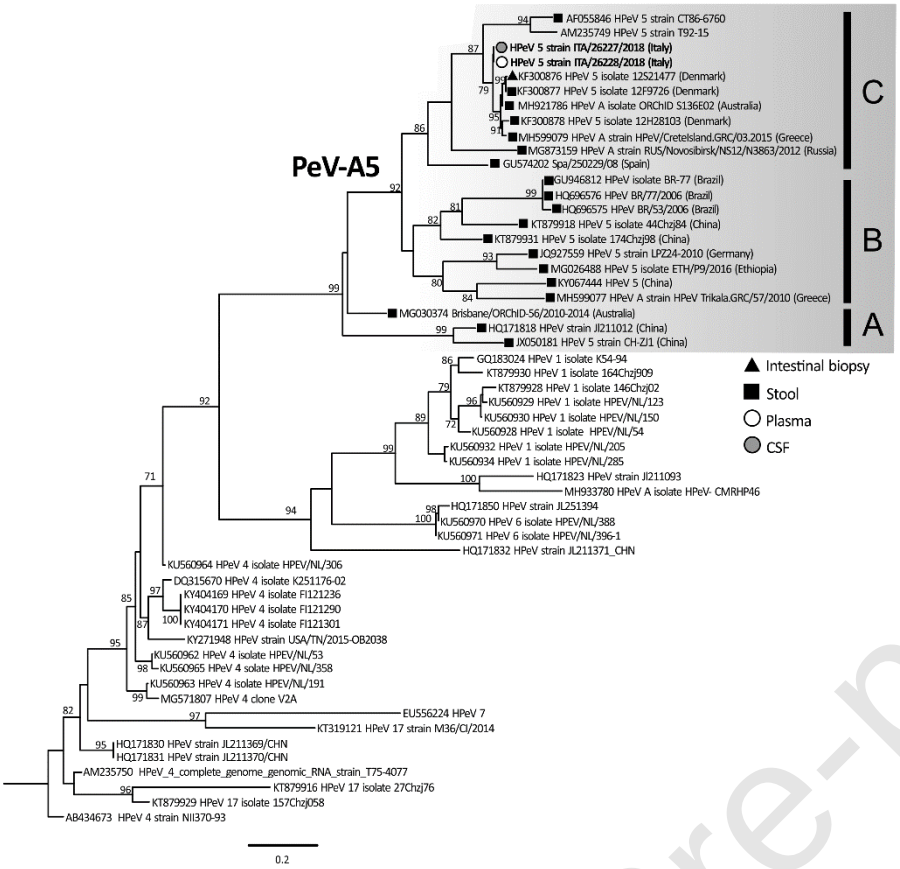


**Figure 1.** Phylogenetic rooted tree inferred by maximum likelihood of partial VP3/VP1 sequences (296 nt). Significant branch support values (>70%) are given for maximum-likelihood implementations (IQ-TREE bootstrap support) and are reported near the branch nodes. Strains retrieved from GenBank database using BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with >75% nucleotide identity as compared to the two Italian strains (CSF and plasma) were included in the phylogenetic tree.

**Table 1.** Clinical findings, laboratory testing and other investigations performed during the 7 days of hospitalization.

| *Follow-up (days)* | *Signs and symptoms* | *Laboratory testing* | *Other investigations* |
|---|---|---|---|
| 0[a] | → Irritability +++<br>→ fever ++<br>→ fussing ++<br>→ persistent crying ++<br>→ difficultly in feeding++<br>→ axial hypotonia + | → Leukocyte count: 4.4x $10^9$ cells/L with N 34%, L 41%, Mo 24%, Hb 16.2 g/dL, PLT: 419 x $10^9$ cells/L<br>→ CRP :13,8 mg/L<br>→ CSF: clear colorless appearance, glucose: 52 mg/dl, total protein 49 mg/dl, cells: absent<br>→ Fecal antigen assays (rotavirus, adenovirus, norovirus, astrovirus, sapovirus): negative<br>→ Blood, urine and CSF: sent for culture | |
| 1 | → Fever ++,<br>→ Irritability ++,<br>→ plaintive crying + | → CRP: 12,9 mg/L | Negative Cranial ultrasound |
| 2 | → Fever + | → HSV 1-2 DNA in CSF: negative<br>→ Urine culture: negative | |
| 3 | → Fever + | | |
| 4 | → Defervescence | | |
| 5 | → Improving, good breastfeeding | → CSF culture: negative<br>→ Blood culture: negative<br>→ **PeV real time RT-PCR on blood: positive**<br>→ **PeV real time RT-PCR on CSF: positive** | |
| 6 | → Improving, good breastfeeding | → Leukocyte count: 8.62 x $10^9$ cells/L with N 16%, L 73%, Mo 8%, Hb 17.3 g/dL, PLT: 361 x $10^9$ cells/L<br>→ CRP: 0,7 mg/L | |
| 7[b] | → negative neurological examination, mild impairment in visual fixation, mild axial hypotonia | | |

+ Mild; ++ moderate; +++ severe; N: neutrophils; L: lymphocytes; Mo: monocytes; Hb: hemoglobin; PT: platelet; CRP: C-reactive protein; HSV-1 e 2: herpes simplex virus 1 and 2;

[a] Day of admission

[b] Day of discharge