Detection and identification of human parechoviruses from cerebrospinal fluid specimens

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Human parechoviruses (HPeVs) are associated with clinical manifestations ranging from asymptomatic diseases to infections of the central nervous system. Neonates are considered at risk for severe infections. HPeVs share these features with enteroviruses.

Although several methods for detecting the HPeV genome in clinical specimens have been developed, diagnosis of HPeV infections – especially for differential diagnosis of sepsis-like illness in young children – is not implemented in the routine practice of clinical virology laboratory yet.

Objectives

1) To evaluate the performance of two diagnostic methods : a classic in-house RT-PCR assay and a commercial real-time Parechovirus r-gene ™ assay (*see below*)

2) Retrospective screening of EV-negative cerebrospinal fluid (CSF) samples to determine the prevalence of HPeV infections and associated types.

Patients and methods

• Comparison of the relative detection limit for the prototype strains of HPeV 1 et 2 with two methods, using 10-fold dilution series from 10^{-1} to 10^{-8}.

Method 1: classic in-house RT-PCR assay (*adapted from the real-time RT-PCR assay described by Nix et al., 2008*)
Method 2: real-time Parechovirus r-gene assay (bioMérieux/Argène, France)

• Patients

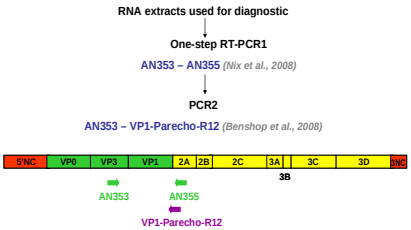
CSF specimens were collected from patients admitted for suspicion of meningitis in the University Hospital of Clermont-Ferrand, France

=> 100 CSF samples (collected between March, 1st and December, 18th 2010) were tested comparatively with both methods

- 67 children (2 days – 16 years) - 33 adults (20 – 76.5 years)

=> 26 supplementary CSF samples were selected among children under 5 years of age (range : 5 days to 4 years; admitted between May, 1st and September, 30th 2010). They were analysed with method 1 (in-house RT-PCR).

• Direct genotyping by a semi-nested RT-PCR (VP1)



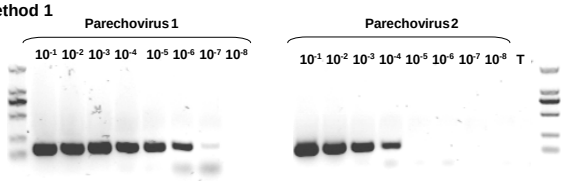
• BLAST analysis was performed by comparison of the obtained sequences with all available sequences of HPeV in Genbank

• Phylogenetic analysis : *Phylogenetic relationships were inferred through genetic distances calculated with the Tamura-Nei model of sequence evolution using the MEGA5 software (Tamura et al., 2011).*

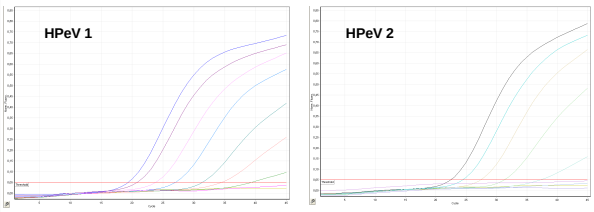
Relative detection limit of the two RT-PCR assays

• The real-time parechovirus r-gene assay (method 2) displayed a sensitivity slightly higher than the classic in-house RT-PCR assay (method 1), especially for detecting HPeV prototype strain type 2.

Method 1



Method 2



Dilution serie	Obtained Ct
HPeV1 10^{-1}	19,37
HPeV1 10^{-2}	20,82
HPeV1 10^{-3}	23,83
HPeV1 10^{-4}	27,01
HPeV1 10^{-5}	31,36
HPeV1 10^{-6}	34,48
HPeV1 10^{-7}	39,23
HPeV1 10^{-8}	Absence

Dilution serie	Obtained Ct
HPeV2 10^{-1}	22,45
HPeV2 10^{-2}	24,45
HPeV2 10^{-3}	27,42
HPeV2 10^{-4}	31,29
HPeV2 10^{-5}	37,26
HPeV2 10^{-6}	Absence
HPeV2 10^{-7}	Absence
HPeV2 10^{-8}	Absence

Figure 1. Detection of HPeV genome in ten-fold dilution series of human parechovirus types 1 and 2 prototype strains with an in-house RT-PCR assay (method 1; Nix et al., 2008) and the real-time parechovirus r-gene kit (method 2; bioMérieux/Argène)

Analysis of Cerebrospinal Fluid Specimens

• 100 CSF specimens were be tested comparatively

• *Inhibitors of PCR were evidenced for two samples with the real-time Parechovirus r-gene ™ kit*

• Concordant results were obtained for 97/98

Table 1. Comparison of the genome detection of HPeV in 98 CSF specimens with the two methods tested

		In-house RT-PCR assay	
		Positive	Negative
Parechovirus r-gene kit	Positive	3	1 *
	Negative	0	94

*** The CSF sample was re-analysed twice and found to be repeatedly positive both times with the in-house RT-PCR assay**

→ HPeV was detected in 4/98 (4.1 %) of the patients

• Among the 26 supplementary CSF samples selected among children under 5 years of age between May and September and analysed with the in-house RT-PCR

→ HPeV was detected in 3/26 (11.5 %) of the patients

Demographical and clinical data of HPeV-infected patients

• HPeV infections all occurred in the summer months (June to August)

Table 2. Clinical characteristics of 7 patients with human parechovirus in CSF

Variable	Finding [a]
Age, days	
Mean	42.1
Median [range]	28 [9-127]
Fever	7/7 (100)
Irritability	4/7 (57)
Sepsis like illness	4/7 (57)
CSF	
Cell count, mean no of cells/mm^3 [range]	1.4 [0-5]
Elevated protein level [b]	3/7 (43)
Gastrointestinal tract symptoms	3/7 (43)
Duration of hospital stays, days	
Mean	5
Median [range]	3 [2-10]
Antibiotic treatment	4/6 (67)

[a] Data are proportion (%) of patients with symptoms for whom data were available

[b] CSF protein level (<0.90 for children under 30 days : <0.5 for older children)

• All patients infected by HPeV were children < 4 months

• Ratio male/female = 5/2 (2.5)

• Pleocytosis (white blood cell count <10/mm^3) was not observed

• All patients presented with fever

• All were discharged to home in good condition

Genotyping and phylogenetic analysis

• HPeV from all 7 positive samples was typed as HPeV type 3 by BLAST analysis

• 6 VP1 sequences of patients were available for phylogenetic analysis

• HPeV3 strains identified in this study (●) displayed close genetic relationships to one another and with strains isolated in the Netherlands between 2004 and 2006.

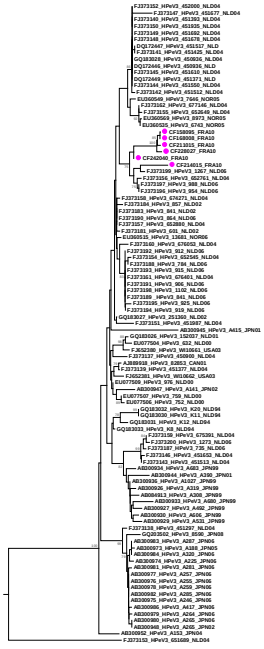


Figure 3. Phylogenetic tree based on 99 partial 1D (VP1) sequences (594 nt) of HPeV3 strains

Phylogenetic tree was constructed by the neighbor-joining method and validated with 1,000 bootstrap pseudo-replicates. Only bootstrap values of over 70 % are shown. The HPeV1 prototype strain (L02971) was used to root the tree. The countries are designated by a three-letter ISO code (ISO 3166).

CAN, Canada; FRA, France; JPN, Japan; NLD, The Netherlands; NOR, Norway; USA, United States of America.

Conclusions

• The real-time RT-PCR assay allows rapid HPeV detection in CSF and is suitable for use in routine practice in a clinical virology laboratory

• As for systematic enterovirus testing in the CSF of children with meningitis, implementing HPeV PCR from children, especially younger ones (< 1 year), would improve the aetiological diagnosis of neonatal sepsis and meningitis.

• As in other studies *(Harvala et al., 2009)*, HPeV type 3 was the predominant type in neonates presenting with sepsis-like or febrile illness.

References

• *Nix WA, Maher K, Johansson ES, Niklasson B, Lindberg AM, Pallansch MA, Oberste MS.* ***2008.*** *Detection of all known parechoviruses by real-time PCR. J Clin Microbiol 46: 2519-2524.*

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• *Harvala H, Robertson I, Chieochansin T, McWilliam Leitch EC, Templeton K, Simmonds P.* ***2009.*** *Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. J Infect Dis 199: 1753-1760.*