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Prospective assessment of clinical symptoms associated with enterovirus and parechovirus genotypes in a multicenter study in Dutch children

S.C.M. de Crom[a,b,*], J.W.A. Rossen[c,d,e], R.A. de Moor[a,f], E.J.M. Veldkamp[g], A.M. van Furth[h], C.C. Obihara[a]

[a] Department of Pediatrics, St. Elisabeth Hospital, Tilburg, The Netherlands
[b] Department of Pediatrics, Maastricht University Medical Centre, Maastricht, The Netherlands
[c] Laboratory of Medical Microbiology and Immunology, St. Elisabeth Hospital, Tilburg, The Netherlands
[d] Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, The Netherlands
[e] Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, The Netherlands
[f] Department of Pediatrics, Tweesteden Hospital, Tilburg, The Netherlands
[g] Department of Pediatrics, Amphia Hospital, Breda, The Netherlands
[h] Department of Pediatric Infectious Diseases Immunology and Rheumatology, VU Medical Centre, Amsterdam, The Netherlands

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ABSTRACT

Background: Human non-polio enterovirus (EV) and human parechovirus (HPeV) are important pathogens of viral infection and aseptic meningitis in children. The aim of this study is to prospectively compare the incidence, clinical signs, blood and cerebrospinal fluid in EV and HPeV infected children.
Objectives: To compare the clinical symptoms and laboratory data of children with different EV and HPeV genotypes.
Study design: This study is part of a multicenter prospective cohort study. Children were included in 3 different hospitals in The Netherlands from 2008 to 2011.
Results: Of 285 included patients, 140 (49%) had EV and 44 (15%) HPeV infection. Of children with EV infection 9 (6%) had EV-A, 109 (78%) EV-B, 12 (9%) had a non-type able EV and in 10 (7%) no genotyping was performed. Of children with HPeV infection, 24 (55%) had HPeV-3, 6 (14%) HPeV-1, 2 (5%) HPeV-4 and 1 (2%) HPeV-6. Meningitis was more frequent in EV than in HPeV infected children (54% vs. 36%, $p = 0.046$), and in EV-B than EV-A infected children (60 vs. 33%). In contrast gastroenteritis was more frequent in HPeV than EV infected children (30% vs. 15%, $p = 0.030$), and significantly more in HPeV-1 than HPeV-3 infected children ($p < 0.001$).
Conclusions: EV infection is more often associated with meningitis and HPeV infection more often with a gastro-enteritis. EV genotype B infection is more often associated with meningitis than EV genotype A infection. HPeV-1 infection was more often associated with gastroenteritis than HPeV-3 infection.

1. Background

Human non-polio enterovirus (EV) and human parechovirus (HPeV) are major causes of viral infection and aseptic meningitis, especially in neonates and young infants [1–4]. The clinical spectrum of EV and HPeV infections varies from fever to severe systemic disease, including sepsis and meningoencephalitis, which may result in severe neuropsychological sequelae [5–7]. Recently several clusters of severe respiratory illness resulting in intensive care admissions and fatal outcomes have been attributed to EV type 68 infections across the US [8]. EVs have been recently classified in

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; CV-A, human coxsackievirus A; CV-B, human coxsackievirus B; E, human echovirus; EV, Human non-polio enterovirus; HPeV, human parechovirus; RNA, ribonucleic acid; RT-qPCR, reverse-transcriptase real time quantitative polymerase chain reaction; tMK, Cynomolgus monkey kidney.

* Corresponding author at: Department of Pediatrics, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, The Netherlands. Fax: + 31 13 5359433.
E-mail address: stephanie_de_crom@hotmail.com (S.C.M. de Crom).

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Table 1
Inclusion- and exclusion criteria.

Inclusion criteria
Children ≤16 years of age with any of the following clinical signs and symptoms: 1. Fever (temperature ≥38.0 °C or ≥100.4 °F) or 2. Clinical sign or symptom of meningitis: headache, photophobia, nuchal rigidity, irritability, lethargy, nausea, vomiting, drowsiness, positive sign of Kernig or Brudzinsky[a] or 3. Other clinical signs and symptoms: hypothermia, headache, drowsiness, nuchal rigidity, irritability, photophobia, vomiting, diarrhea, anorexia, coughing, myalgia, rash[b] or 4. Clinical sign(s) of sepsis and - Signed informed consent by the parent(s)/legal guardian(s)
Exclusion criteria
- Other proven infectious cause of the clinical symptoms - Other non-infectious cause of clinical symptoms: e.g. neoplasm, auto-immune diseases, rheumatic diseases, endocrinologic diseases, gastro esophageal reflux, etcetera - Patients ≥16 years of age - No parental consent

[a] At least 2 of these signs or symptoms must be present.
[b] At least 3 of these signs or symptoms must be present.

types EV-A (25 genotypes), EV-B (63 genotypes), EV-C (23 genotypes) and EV-D (5 genotypes). HPeV has been classified into 16 different genotypes according to the standard definition [9].The aim of this study is to prospectively compare the incidence, clinical signs blood and cerebrospinal fluid (CSF) changes in EV and HPeV infected children.

2. Objectives

To compare the clinical symptoms and laboratory data of children infected with different EV and HPeV genotypes.

3. Study design

This study is a part of a multicenter prospective cohort study to evaluate the incidence, clinical features and prognosis of EV and HPeV infection in children. We compared the clinical symptoms and laboratory data of children infected with different EV and HPeV genotypes at inclusion. The study was conducted in two of the largest non-university teaching hospitals (St. Elisabeth Hospital in Tilburg and Amphia Hospital in Breda) and in a non-teaching hospital (Tweesteden Hospital in Tilburg) in The Netherlands between 1st of March 2008 and 30th of September 2011.

3.1. Patients and enrolment

Children aged 0–16 years, with clinical suspicion of a viral infection were eligible and only those with confirmed EV or HPeV infection were included in the present study. The study enrolment has been extensively described previously [10,11] and are included in Table 1. Following inclusion, nasopharyngeal, blood, urine and feces specimens were collected for EV and HPeV reverse-transcriptase real time quantitative polymerase chain reaction (RT-qPCR), and feces and nasopharyngeal specimens for viral culture. If the pediatrician clinically suspected the child to have meningitis or meningoencephalitis, a lumbar puncture was performed accordance to routine clinical practice and CSF specimen collected for EV and HPeV RT-qPCR and viral culture, in addition to bacterial culture. Children were excluded if they were older than 16 years of age, another viral, bacterial or fungal pathogen identified or a non-infectious reason found for cause of clinical presentation or with non-consenting parents.

3.1.1. Viral RNA isolation

An aliquot (200 μl) of nasopharynx, blood, urine, feces or CSF specimen was used to extract viral RNA using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Basel, Switzerland) as described previously [10,11].

Each sample was eluted in 50 μl buffer. All samples were spiked before extraction with an internal control virus (phocine distemper virus) to monitor for efficient extraction and amplification, as described previously [12].

3.1.2. RT-qPCR

The isolated viral RNA was reverse transcribed using MultiScribe RT and random hexamers (both from Applied Biosystems, Foster City, CA l). Detection of EV and HPeV was performed using an EV specific and HPeV specific RT-qPCR as previously described [13]. Real-time PCR procedures were performed as described previously [10,13]. The detection rates of human EV and HPeV RT-qPCR and viral culture in the different pediatric specimens were described previously [11].

3.1.3. Genotypes EV and HPeV

Genotyping of EV and HPeV was performed as described previously by Nix et al. and Harvala et al., respectively [14,15]. Genotypes were assigned by blasting obtained sequences against known sequences in Genbank using the NCBI blast tool. EV was classified into species A (EV-A), B (EV-B), C (EV-C) and D (EV-D) according to the standard definitions. Each species consisted of multiple genotypes. HPeV can be genotyped into 16 different types according to the standard definition [9]. Sequences have been deposited at GenBank under the accession numbers KU560921–KU561064.

3.1.4. Viral culture

Viral culture was performed on confluent layers of tertiary Cynomolgus monkey kidney (tMK) cells. After inoculation of 0.25 ml of clinical specimen and absorption in the cells for 1 h, 1 ml of culture medium was added and cells, maintained at 37 °C on roller drums, were examined daily during 14 days for a cytopathic effect. Serotyping of the virus isolates was carried out by neutralization or complement fixation with intersecting antiserum pools by standard procedures.

3.2. Definitions

EV or HPeV infection was defined as the detection of EV or HPeV in viral culture or RT-qPCR of nasopharyngeal, blood, urine, feces or CSF specimens of a symptomatic patient.

EV or HPeV meningitis was defined as the detection of EV or HPeV in the CSF of a symptomatic patient.

EV or HPeV gastroenteritis was defined as the detection of EV or HPeV in the feces of a symptomatic patient (Table 1).

3.3. Data collection and statistical methods

Data on demographics, presenting symptoms, duration of symptoms, hospitalization, antibiotic prescription, results of blood and CSF chemistry, results of viral culture or RT-qPCR and completed standardized questionnaires were captured in a Microsoft Access 2007 database.

Statistical analyses were performed with SPSS 22.0 (Windows Inc., Chicago, USA). Chi-squared test or the Fisher's exact test was used for the analysis of categorical data and the student *t*-test for continuous variables with normal distribution. The Mann–Whitney