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Viral infections in young infants : epidemiologic and diagnostic aspects of ToRCH, enterovirus and human parechovirus

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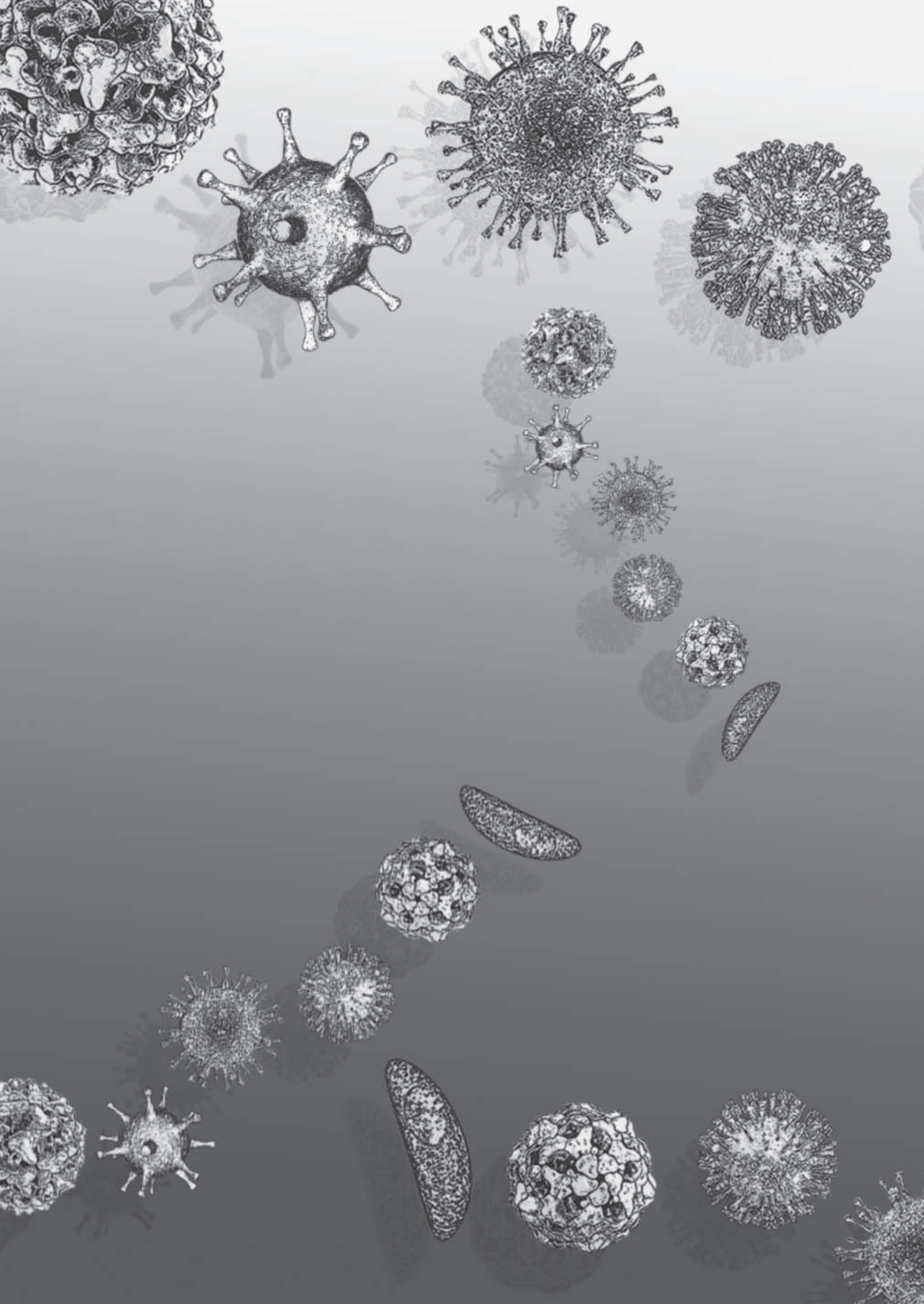


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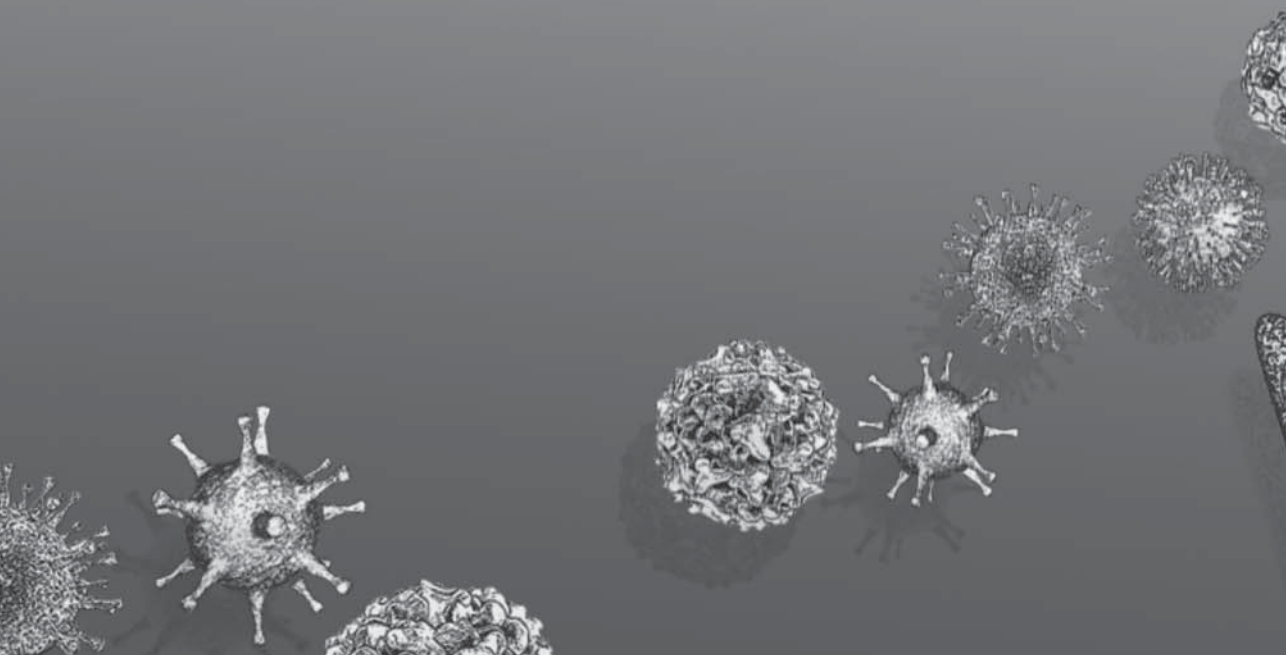
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PART B

Enterovirus and Human Parechovirus



5

**EPIDEMIOLOGY OF SEPSIS-LIKE ILLNESS IN
YOUNG INFANTS: MAJOR ROLE OF ENTEROVIRUS
AND HUMAN PARECHOVIRUS**

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ABSTRACT

Background: Sepsis-like illness is a main cause for hospital admission in young infants. Our aim was to investigate incidence, epidemiology and clinical characteristics of enterovirus (EV) and human parechovirus (HPeV) infections in young infants with sepsis-like illness.

Methods: This is a prospective observational cohort study in which infants less than 90 days of age, presenting with sepsis-like symptoms in a secondary care children's hospital, underwent a full sepsis work-up. Clinical signs and infectious indices were recorded. EV or HPeV RNA was detected by PCR in plasma and/or cerebrospinal fluid (CSF).

Results: Infants were diagnosed with EV, HPeV, fever of unknown origin or severe infection. EV and HPeV were detected in 132/353 (37%) and 52/353 (15%) of cases. EV and HPeV have distinct seasonability. Some differences in clinical signs and symptoms occurred between children with EV and HPeV infection, but were of limited clinical value. CSF pleocytosis occurred in 44% of EV positive infants, and only in 13% of those with HPeV infection.

Conclusions: EV and HPeV infections are major causes of sepsis-like illness in infants < 90 days of age. Neither clinical characteristics nor laboratory indices were predictive for EV/HPeV infection. CSF pleocytosis occurs, but not in all patients. Testing for EV and HPeV in all young infants with sepsis-like illness is strongly advised.

INTRODUCTION

Sepsis-like symptoms in children, especially in young infants (under 90 days of age) remain a diagnostic challenge for paediatricians because it is often hard to distinguish between serious bacterial infections and more benign viral infections^{1,2}. In young infants enterovirus (EV) and human parechovirus (HPeV) infections are a known cause of sepsis-like illness, aseptic meningitis and febrile disease³⁻⁶. Numerous EV types, specifically several serotypes of the enterovirus B species, have been associated with febrile-illness and aseptic meningitis in infants^{7,8}. In HPeV infection, type 3 (HPeV3) is the main genotype causing sepsis-like symptoms in young infants^{9,10}. EV and HPeV infections can also cause serious symptoms such as cardiorespiratory instability and neurologic symptoms, leading to hospital or, in some young infants, Paediatric Intensive Care Unit admittance¹¹⁻¹⁴.

Previous studies have reported a high incidence of EV and HPeV infections among febrile infants, but most were retrospective^{15,16}, based on laboratory¹⁷⁻¹⁹ results rather than clinical presentation, did not solely focus on young infants³ or described neonates only⁶. Only one prospective cohort that included patients up to 90 days of age was described earlier⁵.

We performed a prospective observational cohort study to describe epidemiology, clinical characteristics and infectious indices of young infants with sepsis-like illness who presented at our emergency department. Our hypothesis is that EV and HPeV are a major cause of sepsis-like illness in this vulnerable group of infants up to 90 days of age and that symptoms of infants with EV or HPeV infection are not different from other infants with sepsis-like illness. Main outcome is frequency of diagnosis of EV or HPeV infection in our study population, secondary outcomes are clinical signs and symptoms and laboratory indices.

MATERIALS AND METHODS

Study protocol

This prospective observational cohort study was performed at the Juliana Children's Hospital, The Hague, Netherlands. All children under 90 days of age who were evaluated at our emergency department for sepsis-like symptoms between January 1, 2008 and June 30, 2012 were evaluated in this study. Sepsis-like illness was diagnosed based on age-specific criteria (Table 5.1), which were evaluated at physical examination by the attending physician. All physicians in our hospital were trained in their use. In addition to the clinical signs and symptoms described in Table 5.1, the following clinical parameters were collected: sex, prematurity (gestational age < 37 weeks), medical history, abnormal behaviour (defined as lethargic or agitated), skin rash, oxygen saturation at presentation and duration of symptoms

Table 5.1: Criteria for sepsis-like illness according to age*

Age at presentation	0–28 days	1–3 months
Clinical signs and symptoms	One or more: - toxic appearance - temp < 36.0 °C or > 38.0 °C - feeding problems - lethargy or agitation - tachypnea - tachycardia - capillary refill > 2 sec	One or more: - toxic appearance - temp < 36.0°C or > 39.0°C - fever > 48 hours - lethargy or agitation - capillary refill > 2 sec - bulging fontanel
Criteria for toxic appearance	Rochester criteria ²⁰	Yale observation scale ²¹ > 10

* Local adaptation of national guidelines for management of children with fever without source (Dutch Association of Paediatrics, NvK).

before presentation. If a specific symptom was not clearly noted on admittance, this item was labelled as ‘missing’.

We excluded patients with signs of a localized infection, defined as clinically apparent gastroenteritis, upper respiratory tract infection, pneumonia (clinically apparent and confirmed on chest x-ray) or abnormal analysis of urine sediment (more than five white blood cells (WBC) per microscopic field view, magnification of 40 times).

Patients with need of systemic intravenous treatment for a confirmed (with bacterial culture or HSV PCR) pathogen were assigned to the group ‘severe infection’. Patients with insufficient sample size of both plasma and cerebrospinal fluid (CSF) to perform PCR for EV and HPeV were labelled as ‘non-evaluable result’. To investigate whether or not these patients influenced our results, we performed additional analyses including them once in the EV or HPeV group and once in the fever of unknown origin (FUO) group.

Biochemical and microbiological data

Children underwent blood and CSF sampling for biochemical analysis, viral analysis for EV and HPeV, and bacterial cultures. Herpes simplex virus PCR was performed on CSF.

The results for C-reactive protein, serum glucose, full blood count, and WBC differentiation were recorded. When CSF was successfully collected, CSF white and red blood cell counts, CSF glucose and protein levels were recorded in the study database. We corrected CSF WBC count for traumatic puncture if the CSF red blood cell count was > 1000 cells/μL, using a 1000:1 ratio ²⁰. CSF pleocytosis was defined as a CSF WBC count > 19 cells/μL for children < 28 days of age, > 9 cells/μL for children 28–58 days of age and > 5 cells/ μL for children 59–90 days of age ^{21 22}.

EV and HPeV detection and genotyping

PCR was performed on plasma and CSF to detect EV or HPeV RNA. RNA was extracted from 200 µL of plasma and/or 50–200 µL of CSF with the Nuclisens easyMAG system (Biomérieux, Boxtel, Netherlands). The manufacturer's protocol (Generic 1.0) was followed using easyMAG specific reagents. A fixed amount of Phocine Distemper Virus served as an internal control and was added to each sample prior to RNA extraction. Extracted RNA was used for Reverse Transcription PCR to synthesize copy DNA and PCR was performed with the ABI 7500 Real Time PCR system (Applied Biosystems, USA): 10 minutes at 95°C, followed by 45 cycles of 15 sec at 95°C, and 1 minute at 60°C. Primers and probes for amplification and detection of EV and HPeV were located in the highly conserved 5' end of the genome. Modifications were made to previously described probes and primers to also detect HPeV 3²³⁻²⁵. Probes were adjusted to VIC-TTACCTR CGGGTACCTTCTGGGCATCCTT-TAMRA and VIC-CCCCAGATCAGATCC-MGB and primers were adjusted to TGCAAACACTAGTTGTAAGGCC, TGCAGACACTAGTTGTAAGGCC, TGCAAACACTAGTTGTATGGCCC (forward primers) and TTGGCCCACTAGACGTTTTTAA, TTGGCCCGCTAGACGTTTTTAA, GTTTGGCCCACTAGACGTTTTT (reverse primers).

All PCR runs had a mixture of an EV and HPeV strain as a positive control and nuclease free water as a negative control. A positive diagnosis for infection with EV or HPeV was made on a positive PCR in either plasma or CSF (or both).

EV and HPeV positive plasma and CSF samples were genotyped in one batch after completion of the study period if enough material was left. EV typing was performed as previously described with modifications²⁶. In short, two PCR's were run (EV-A and EV-B) for which 6 µL of input RNA was used. The original protocol was adjusted to perform a semi-nested PCR instead of a single PCR¹⁹. PCR-1 was performed using primers (EV-A OS 2268 + EV-A OAS 3109 and EV-B OS 2324 + EV-B OAS 3505) for 1 hour at 43°C, followed by 2 x 20 cycles at 53 and 55°C, 15 minutes at 72°C, and 2 minutes at 94°C. Thereafter, 3 x 40 cycles were performed at 94°C, 50°C and 68°C, followed by 5 minutes at 68°C. One µL of this fluid was then transferred to PCR-2 with primers (EV-A OS 2268 + EV-A IAS 3016 and EV-B OS 2324 + EV-B IAS 3477). This was processed in 3 x 30 cycles (18 min at 94°C, 21 min at 55°C and 90 min at 72°C), followed by 5 min at 72°C. Fluid of PCR-2 (5–10 µL) was loaded on an agarose gel, if positive (band visible of 750bp (EV-A) or 1150bp (EV-B)), a standard BDT sequence reaction was performed using primers for PCR-2²⁶.

HPeV typing was performed as previously described by Harvala et al.¹⁹. One modification was made; we used a different OS primer (HPeV OS-R-2162; TCMACWTGGATGAGGAARAC instead of the original primer HPeV OS-2090) in PCR-1.

Statistical analysis

SPSS was used for data management (PASW statistics version 17.0) and statistical analysis (IBM SPSS statistics version 23.0). Data were checked for normality before analysis, using descriptive statistics and histograms with z-scores for skewness and kurtosis. Categorical data are shown as absolute number/total (percentage) and numerical data as median (interquartile range). P-values < 0.05 were considered to indicate statistical significance, in subgroup analyses we considered p-values < 0.01 statistically significant. Mann-Whitney-U tests and Kruskal Wallis tests were used for numerical data and Fisher's Exact tests for categorical data. Binary logistic regression analysis was performed to assess the relationship between the occurrence of EV or HPeV infection or FUO (dependent variable) and clinical characteristics or laboratory parameters (independent variables).

The data described in our study were derived from our standard of care. No extra interventions were conducted for study purposes only. Therefore, no explicit informed consent from parents was warranted for this study. The personal data of our patients were protected. The study was approved by the regional medical ethics committee.

RESULTS

During the study period 362 infants with sepsis-like illness were included. Nine infants (2%) could not be diagnosed due to insufficient sample volume of either plasma or CSF to perform EV and HPeV PCR. The additional analyses to investigate the influence of these non-evaluable patients to our cohort showed no change in our results (data not shown).

Epidemiology

The remaining 353 infants were diagnosed as: EV infection (n = 132 (37%)), HPeV infection (n = 52 (15%)), fever of unknown origin (FUO) (n = 162 (46%)) and severe infection (n = 7 (2%)). Details of the recruitment and diagnoses of our cohort and the causative pathogens of infants in the 'severe infection' group are given in Figure 5.1.

Figure 5.2 shows the seasonal distribution of the different diagnoses. During summer, there is a yearly peak of EV infection and biannual peak of HPeV infections in even years. During winter 2009 an increase in FUO occurred during the influenza A (H1N1) pandemic in our country. Bacterial infections occurred with a low incidence throughout the study period.

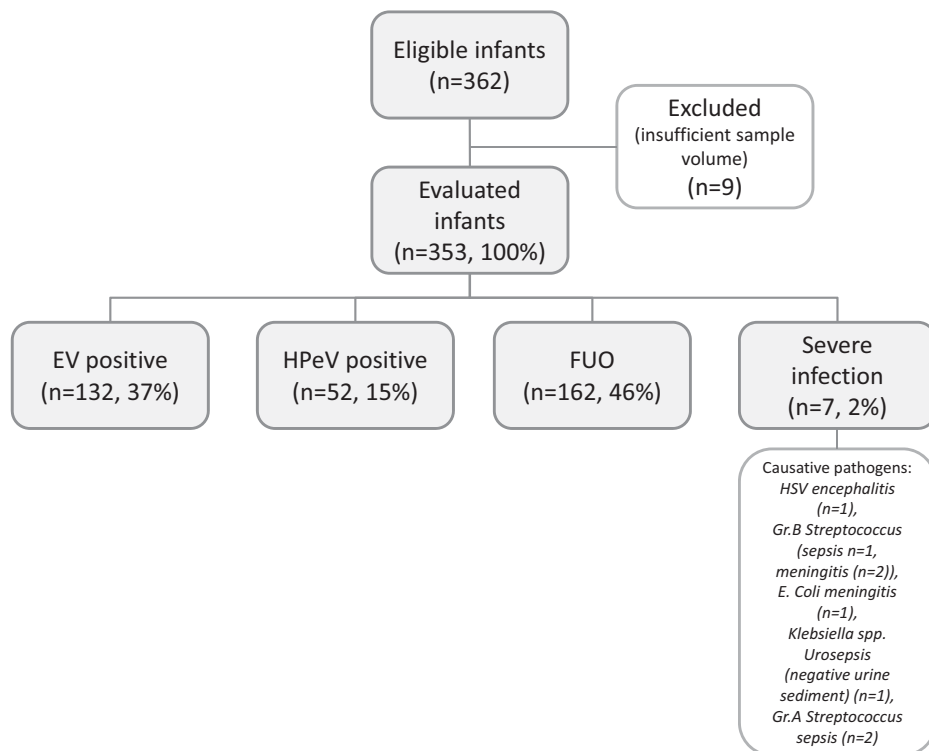


Figure 5.1: Flowchart of study-population and details of causative pathogens of those in the severe infection group.

Viral genotyping

Enough material was available to perform genotyping in 35/184 EV or HPeV positive infants (19%). Genotyping was possible for 23/132 (17%) EV positive patients, of whom 22 were enterovirus-B positive (CV-B1, CV-B2, CV-B4, CV-B5, E-6, E-7, E-9, E-11, E-18, E-25, and E-30) and 1 enterovirus-A (CV-A16) positive. This infant presented with sepsis-like illness, was EV positive in plasma and negative in CSF, and did not develop any signs of hand-foot-mouth disease during its hospital stay. Table 5.2 shows the details of EV genotyping. HPeV-3 was found in all of the HPeV positive samples that were genotyped (12/52 (23%)).

Clinical and biochemical parameters

Clinical symptoms, vital parameters and infectious parameters of our study population are presented in Table 5.3 and 5.4. No statistically significant differences occurred between the serious bacterial infections group and the FUO and 'EV or HPeV' group.

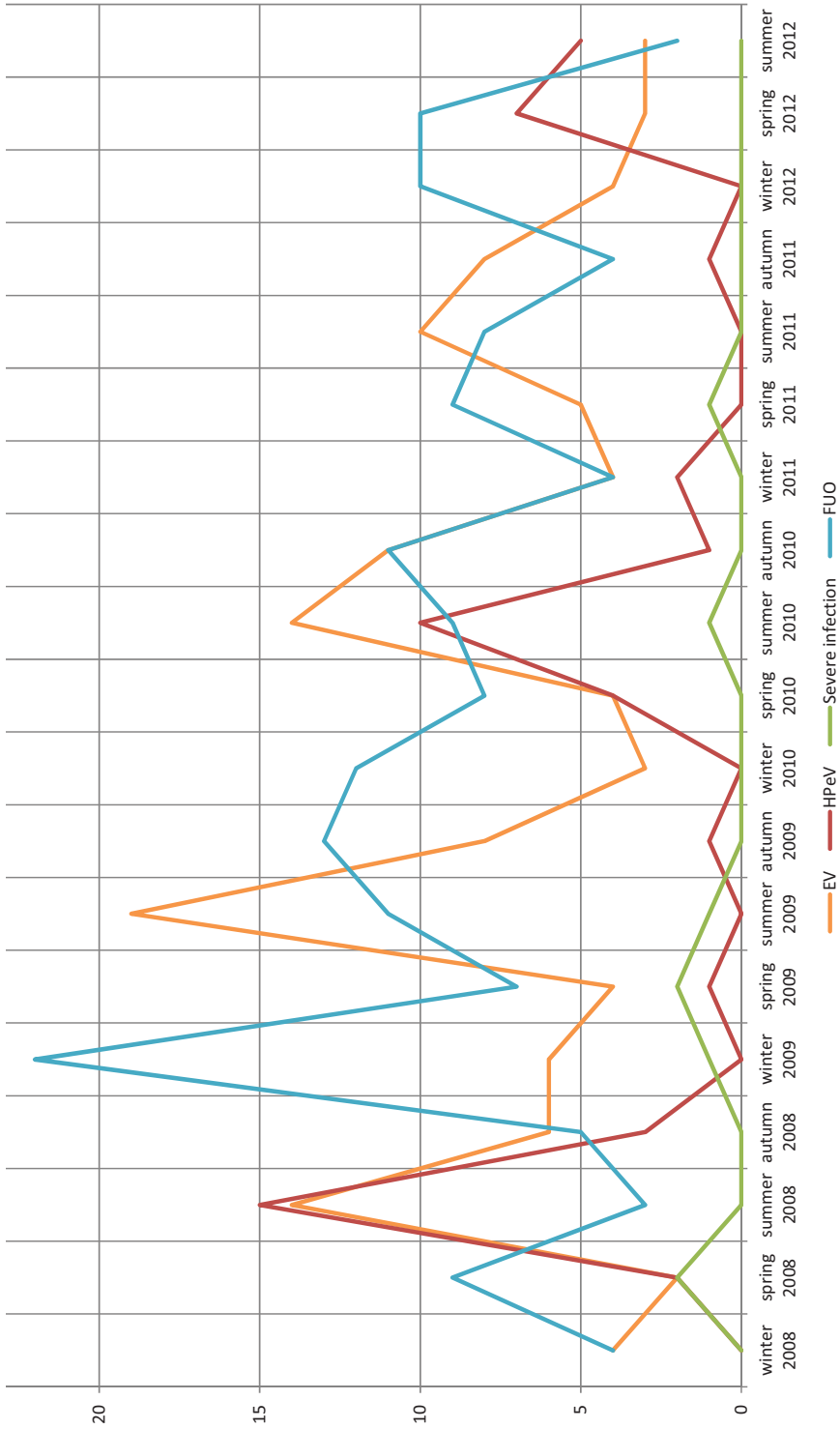


Figure 5.2: Seasonal distribution per diagnosis.

Black = EV; dotted = HPeV; dark gray = severe infection; light gray = FUO.

Table 5.2: Enterovirus serotyping details

Species	Serotype	Number of patients	Season/year of diagnosis
Enterovirus B	E-25	3	Winter, Spring, and Summer 2008
Enterovirus B	E-7	1	Summer 2008
Enterovirus B	CV-B4	1	Summer 2008
Enterovirus B	CV-B1	2	Summer and Autumn 2008
Enterovirus B	E-9	3	Summer and Autumn 2009
Enterovirus B	CV-B5	3	Winter, Spring and Summer 2009
Enterovirus B	E-6	2	Summer 2009
Enterovirus B	E-11	2	Winter 2009 and Summer 2010
Enterovirus B	CV-B2	2	Summer and Autumn 2010
Enterovirus B	E-30	2	Summer 2010 and Winter 2011
Enterovirus A	CV-A16	1	Autumn 2010
Enterovirus B	E-18	1	Summer 2012

Table 5.3: Clinical characteristics and laboratory indices of total study population

	Severe infections [^] (n = 8, 2%)	EV or HPeV* (EV, n = 132, 37%) (HPeV, n = 52, 15%)	FUO* (n = 161, 46%)	p-value
Sex (males)	6/8 (75%)	116/184 (62%)	85/161 (52%)	0.063
Positive medical history	1/8 (13%)	9/184 (5%)	10/161 (6%)	0.641
Prematurity	1/6 (17%)	6/165 (5%)	7/148 (5%)	1.000
Age < 28 days	4/8 (50%)	101/184 (55%)*	62/161 (39%)*	0.003
Rash	2/8 (25%)	29/178 (16%)	23/154 (15%)	0.764
Duration of illness before presentation	0.5 (0.1–2.5)	0.5 (0.5–1.0)	0.5 (0.5–1.5)	0.450
Body temperature (°C)	38.7 (38.0–39.1)	38.7 (38.3–39.1)*	38.5 (38.1–38.9)*	0.001
Heart frequency (/min)	164 (147–187)	172 (158–188)*	167 (150–180)*	0.007
Breathing frequency (/min)	37 (32–56)	50 (40–60)*	44 (35–52)*	0.001
Oxygen saturation (%)	99 (97–100)	100 (98–100)	99 (98–100)	0.134
Capillary refill > 2 sec (%)	3/7 (43%)	46/177 (26%)	32/155 (21%)	0.299
Abnormal behaviour	5/8 (63%)	140/182 (77%)*	102–161 (63%)*	0.006
White blood cell count (x 10 ⁹ /L)	14.0 (3.9–18.5)	7.7 (5.6–10.1)*	10.1 (8.0–14.3)*	0.000
Blood neutrophil count (x 10 ⁹ /L)	8.6 (1.6–12.0)	3.6 (2.2–4.8)*	4.8 (2.8–7.0)*	0.000
C-reactive protein (mg/L)	22 (7–41)	6 (3–20)	6 (3–19)	0.964
Pleocytosis (%)	3/8 (38%)	51/152 (34%)	17/91 (19%)	0.013
CSF white blood cell count (x/3µL)	13 (5–359)	8 (2–58)*	3 (2–11)*	0.003
CSF glucose (mmol/L)	2.4 (1.0–3.1)	2.9 (2.6–3.3)	3.1 (2.8–3.3)	0.044
CSF protein (mg/L)	0.79 (0.55–1.58)	0.54 (0.41–0.72)	0.49 (0.34–0.68)	0.087

P-values < 0.01 were considered to indicate statistical significance (subgroup analysis) Mann-Whitney-U tests were used for numerical data and Fisher's Exact tests for categorical data.

[^] No statistically significant difference existed between the severe infection and other subgroups.

* P-values indicate a difference between the 'EV or HPeV' and 'FUO' groups.

Table 5.4: Comparison of EV and HPeV positive infants

	EV (n = 132)	HPeV (n = 52)	p-value
Sex (males)	84/132 (64%)	32/52 (62%)	0.866
Positive medical history	7/132 (5%)	2/52 (4%)	1.000
Prematurity	5/126 (4%)	4/48 (8%)	0.263
Age < 28 days	77/132 (58%)	24/52 (46%)	0.143
Rash	19/127 (15%)	10/51 (20%)	0.502
Duration of illness before presentation	0.5 (0.5–2.0)	0.5 (0.25–1.0)	0.013
Body temperature (°C)	38.6 (38.3–38.9)	38.8 (38.3–39.1)	0.223
Heart frequency (/min)	170 (156–185)	182 (161–195)	0.012
Breathing frequency (/min)	50 (40–60)	48 (40–59)	0.447
Oxygen saturation (%)	100 (98–100)	100 (98–100)	0.469
Capillary refill > 2 sec (%)	28/128 (22%)	18/49 (37%)	0.055
Abnormal behaviour	96/131 (73%)	44/51 (86%)	0.078
White blood cell count (x 10 ⁹ /L)	8.2 (6.6–10.8)	5.2 (4.0–8.1)	0.000
Blood neutrophil count (x 10 ⁹ /L)	4.0 (2.7–5.1)	2.3 (1.6–3.6)	0.000
C-reactive protein (mg/L)	8 (3–24)	5 (2–9)	0.027
Pleocytosis (%)	48/113 (43%)	3/39 (8%)	0.000
CSF white blood cell count (x/μL)	13 (3–151)	4 (2–9)	0.001
CSF glucose (mmol/L)	2.8 (2.6–3.2)	3.2 (2.9–3.4)	0.000
CSF protein (mg/L)	0.54 (0.43–0.74)	0.47 (0.38–0.64)	0.149

P-values < 0.01 were considered to indicate statistical significance (subgroup analysis). Mann-Whitney-U tests were used for numerical data and Fisher's Exact tests for categorical data.

Comparing the EV or HPeV group to the FUO group showed that infants in the EV or HPeV positive group were more often less than 28 days of age ($p = 0.003$) and showed statistically significant, but only slightly higher heart and breathing frequencies at presentation compared to patients in the FUO group. There also was a difference in behavior ($p = 0.006$) and children with EV or HPeV had somewhat lower infectious parameters ($p < 0.01$). Comparing EV/HPeV positive infants to those in the FUO group, logistic regression showed differences in age-group (0–28 days or 29–90 days) (OR 0.243 (95% CI 0.101–0.584)), plasma WBC count (OR 0.743 (95% CI 0.601–0.919)) and CSF WBC count (OR 1.009 (95% CI 1.002–1.015)).

Table 5.4 compares between EV and HPeV positive infants. HPeV positive infants have a lower rate of CSF pleocytosis (8%) than EV positive infants (43%) ($p = 0.000$) and have somewhat lower infectious indices than infants with an EV infection.

No children were transferred to a paediatric intensive care unit and none died. All children visited our outpatient clinic 4–6 weeks after hospital admittance. None of them showed any physical abnormalities at this follow-up visit.

DISCUSSION

We describe a high incidence of EV and HPeV infection in the largest prospective cohort study among infants aged 0–90 days to date. This adds to describing the epidemiology, clinical and laboratory signs, and symptoms of sepsis-like illness in young infants, especially those with an EV or HPeV infection.

Several laboratory-based and retrospective studies have identified EV and HPeV as an important cause of sepsis and/or meningitis in young infants^{9,10,27-29}. Rittichier et al. showed, in a prospective study, an incidence of 20% of EV infection in young infants with fever who underwent a full sepsis work-up⁵. We find a higher incidence of EV infection (36%), this may be due to our selection of a population with a higher risk, as we only included those infants with sepsis-like illness instead of all infants with fever. Cabrerizo et al. recently reported an incidence of 38% for EV infection and 11% for HPeV infection in neonates with fever, sepsis or meningitis⁶. We describe similar incidences, but in a population aged up to 90 days, instead of only neonates, adding to the importance of EV and HPeV testing in this group.

In contrast, in previous laboratory-based reports, the incidence of EV and HPeV infections was much lower^{10,12,28}. For example, Wolthers et al. detected EV in 14% and HPeV in 4.6% of cerebrospinal fluid (CSF) samples of young children (median age 1 month) with sepsis-like illness and meningitis during a 3-year period²⁸. The lower frequencies found in this study may be attributed to the retrospective analysis of randomly submitted samples instead of samples taken prospectively in a selected patient group, as well as the difference in age groups. Also, we tested both plasma and CSF, instead of CSF only.

We made minor modifications to PCR methods described previously²³⁻²⁵, to also detect HPeV3. With this method, detection rate was similar to or higher than previous reports, and genotyping showed only HPeV3, confirming the accuracy of the adjustments.

In Europe HPeV3 occurs in a biannual cycle with a peak in even-numbered years^{28,30}. We also detected a biannual cycle, but with a much higher incidence of HPeV3 in infants with sepsis-like illness. In our population, the incidence of HPeV in epidemic years increased sharply to 19% in 2008, 26% in 2010 and 27% in 2012, and in non-epidemic years dropped to about 2% (both in 2009 and 2011), but HPeV was never completely absent. The higher detection rate of HPeV, which is presumably mainly HPeV3, is most probably due to the heightened awareness of HPeV as a pathogen and subsequent implementation of HPeV3 specific PCR methods just before our study period³⁰.

Because of low sample volumes viral typing was possible only in part of our population, we were able to type 23/132 (17%) of the EV and 12/52 (23%) of the HPeV positive infants.

As expected, in the 23 EV positive patients we found a wide variety of genotypes, all but one were Enterovirus B genotypes. E-5, 6, 11 and 30, and CV-B5, B4 and B1 have been reported to cause sepsis-like illness in young infants^{16 27 31 32}. However this is the first description of E-7, 18 and 25, and CV-B4 to cause sepsis-like illness in infants.

In addition, we are the first to report a CV-A16 related to sepsis-like illness in infants³³. CV-A16 has caused outbreaks of hand-foot-mouth disease³⁴ and rare complications, such as aseptic meningitis or pulmonary oedema have been described in Asia³⁵. It has also been described as a rare cause of fatal infection in infants, with only 4 cases described worldwide³⁶⁻³⁹.

All of the typed HPeV positive infants were HPeV3 positive. This is concordant with previous reports that describe HPeV3 as a main pathogen for sepsis-like illness and aseptic meningitis in young infants⁹. Although we could only test 23% of our study population, we only found HPeV3 and therefore consider this the main HPeV type causing illness in our patient group. Other HPeV types have not been found in our population, these have been described to cause different symptoms^{40 41}.

Although differences between infants with EV or HPeV and those with FUO (Table 5.3 and 5.4) were statistically significant, most likely due to the large sample size of our population, they have a very limited clinical value because the differences of the variables are small and overlapping. It is interesting to notice, however that the infectious indices of HPeV infected infants are somewhat lower than those of infants with EV infection. The clinical presentation of infants with EV and HPeV infection was similar, as has been reported previously¹¹.

In our study we show that although pleocytosis is uncommon in HPeV infection (8%) compared to EV infection (43%), it is not absent. Recently, Cabrerizo et al. described 32 EV positive and 9 HPeV positive neonates and found no CSF pleocytosis in those with an HPeV infection. EV positive patients showed pleocytosis in 19/32 (59%) of cases⁶. Yun et al. showed that EV meningitis occurred without pleocytosis in 68% of neonates. Absence of CSF pleocytosis was associated with a younger age and a shorter time period between onset of disease and lumbar puncture⁴². In accordance with this study, we evaluated a group of very young patients, in whom a lumbar puncture was performed shortly after onset of disease (median, 0.5 days), and find a low number of pleocytosis and high incidence of EV or HPeV (Tables 5.2 and 5.3). Several studies have reported EV and HPeV positive children without CSF pleocytosis who developed neonatal seizures or cerebral white matter abnormalities^{13 14 28}. More research is required to elucidate whether or not CSF pleocytosis is associated with severity of disease, cerebral white matter involvement and neurological sequelae in children with EV and HPeV infections. Testing for EV and HPeV, even in absence of pleocytosis, should be considered standard of care.

Our study has its limitations. We only tested for the presence of EV and HPeV on blood and CSF and did not perform tests to discover viral infections other than EV, HPeV, and herpes simplex virus in our patients. So, we did not determine the influence of other viruses and did not uncover dual infections of infants with an EV or HPeV infection. Our objective was to identify the impact of EV and HPeV on sepsis-like illness in our population of young infants and this lack of testing for other viruses did not influence our outcome. But it would be of interest for further research.

This study adds a large prospective cohort of young infants with sepsis-like illness to current knowledge. We describe similar findings in epidemiology, with a higher detection rate than previously reported, of HPeV in epidemic years. And although less common than in EV infection, HPeV can cause pleocytosis and aseptic meningitis. Testing for EV and HPeV in plasma and CSF should therefore be standard of care in young infants with sepsis-like illness.

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