Viral Central Nervous System Infections in Infants <90 Days Old: A Paediatric Investigators' Collaborative Network on Infections in Canada (PICNIC) Study

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Abstract

Background

The relative contribution of viruses to central nervous system (CNS) infections in young infants is not clear. For viral CNS infections, there are limited data on features that suggest HSV etiology or that are predictors of unfavorable outcome.

Methods

In this cross-sectional study, the Pediatric Investigators Collaborative Network on Infections in Canada identified infants <90 days of age with CNS infection proven to be due to viruses.

Results

Of 174 proven CNS infections, viral causes accounted for 111 (64%) including enterovirus (EV) (N=103; 93%), HSV (N=7; 6%) and human parechovirus (HPeV) (N=1; 1%). All HSV cases and 45 (43%) non-HSV cases presented before 21 days of age. HSV cases were more likely to require ICU admission ($p=0.010$), present with seizures ($p=0.031$) and have extra-CNS disease ($p<0.001$). Three HSV cases (43%) did not have seizures while 4 (57%) HSV and 33 (32%) non-HSV cases lacked cerebrospinal fluid (CSF) pleocytosis.

Conclusions

Viruses account for about half of proven CNS infections in the first 90 days of life. Empiric therapy for HSV should be considered in suspected CNS infections in the first 21 days even in the absence of CSF pleocytosis unless CSF parameters are suggestive of bacterial meningitis.

Background

The prevention of bacterial meningitis by conjugate vaccines has resulted in viruses accounting for an increasing proportion of central nervous system (CNS) disease in childhood.¹ The improvement in viral diagnostics has made this trend more apparent. Previous studies of viral CNS disease were limited by small sample size, included cases where the etiology was not proven or did not focus on infants.²⁻³ The most common viruses associated with CNS infections are enteroviruses (EV), which compared to bacterial CNS infections are less likely to lead to adverse neurologic outcome. By contrast, herpes simplex virus (HSV) CNS infections result in significant morbidity and mortality, especially if acyclovir is delayed.⁴ It is therefore vital that clinicians know what clinical and laboratory features should lead them to start empiric acyclovir.

This was a cross-sectional analysis to identify infants less than 90 days of age with proven CNS infections. We sought to a) determine the relative contribution of HSV and EV to microbiologically-confirmed CNS infections, b) provide a comparative analysis of the epidemiology and outcome of HSV

and non-HSV viral CNS infection, c) describe factors associated with HSV aetiology and d) identify factors associated with unfavorable outcome.

Methods

Study Population and Design:

Seven paediatric academic centres within the Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) retrospectively enrolled hospitalized infants <90 days of age with microbiologically-confirmed CNS infection January 1, 2013 through December 31, 2014. Cases were identified using appropriate codes from the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Canada diagnostic codes for target discharge (Appendix A) and charts were then reviewed. A previous publication described cases of bacterial CNS infection as proven if bacteria was detected from cerebrospinal fluid (CSF) or brain abscess by means of culture or PCR or probable if CSF pleocytosis was present, along with bacterial growth from another sterile site.⁵ For the purposes of this study, we included all proven cases of viral CNS infection based on the identification of a virus in the CSF using viral culture or polymerase chain reaction (PCR) during life or in the brain tissue using PCR at autopsy. All study centres offered routine PCR testing for HSV and EV while only two centres offered HPeV testing during the study period. Cases with coinfection were excluded unless the investigator deemed that a virus was the main pathogen. There were no other exclusion criteria.

Ethics board approval was obtained from all participating centres with the primary approval coming from the Health Research Ethics Board of the University of Alberta (Study number PRO00055909).

Study Definitions:

1) Case classification: a) early onset if diagnosis was made within the first 6 days of life, b) late onset if diagnosis was made day 7 through 29 of life and c) very late onset if diagnosis was made day 30 through 90 of life.

2) Infants were considered to have extra-CNS disease if there was microbiological, clinical or other laboratory findings consistent with viral disease at other sites.

3) Infants who had i) seizures or ii) head imaging suggesting parenchymal involvement were presumed to have encephalitis. All other cases were deemed to have meningitis.

4) Unfavourable outcomes were defined as:

- Neurodevelopmental sequelae (any one of hearing loss, visual impairment, subsequent seizure disorder requiring therapy beyond discharge or developmental delay noted at follow-up) OR
- Death

Data Collection and Analysis:

Demographic, clinical, microbiological, treatment, outcome and follow-up data were extracted from medical records and entered into Research Electronic Data Capture (REDCap) by each participating center.

Descriptive analysis was conducted. Chi-square or Fisher's exact test was used to compare categorical variables and non-parametric tests were used to compare continuous variables (Mann–Whitney U test). Exploratory analysis was conducted using univariate analyses and where sample size allowed, multivariate analyses to identify clinical, laboratory or outcome differences between HSV and non-HSV viral cases were conducted using factors identified as significant in univariate analysis. Additionally, we used univariate analysis to explore potential factors associated with an unfavorable outcome overall. Epi-info version 7 was used for statistical analysis.

Results

Relative contribution of viruses to microbiologically-confirmed CNS infections

There were 174 cases of proven CNS infections in infants < 90 days old, of which 111 (64%) were viral in origin. One case was excluded due to coinfection with group B streptococcus and EV. EV was the most common identified viral pathogen (N = 103; 93%) followed by HSV (N = 7; 6%) and human parechovirus (HPeV) (N = 1; 1%). The HSV cases included 3 with HSV1 (1 with isolated CNS disease, and 2 with disseminated disease) and 4 with HSV2 (1 with isolated CNS disease, and 3 with disseminated disease).

Descriptive analysis of viral CNS infections

Demographics

The median birth weight was 3345 g (range 1690–4900 g) and median gestational age was 37 weeks (range 29–40 weeks). Infants presented at a median age of 23 (range 3–84) days, with 5 cases occurring during the birth hospitalization (all were EV infection on day 3 to day 21 of life in infants born at 31 to 35 weeks GA). HSV cases presented earlier than non-HSV cases (median 14 days versus 25 days of life; $p = 0.02$) (Table 1). Although the vast majority of EV cases occurred during the second half of the year, cases occurred year-round.

Table 1

Comparison of demographic, clinical and outcome features in infants with HSV and non-HSV viral meningitis by univariate analysis

Characteristics		HSV N = 7	Non- HSV N = 104	P- Value ^H
Demographic	Male gender, n (%)	3 (43)	54 (52)	0.71
	Age at onset (d), median (IQR)	14 (6–19)	25 (12.5–32)	0.02
	Overall			1.0
	Subset fulfilling encephalitis criteria [Proportion (%) age < 28d]	16 (6–19) [6/6 (100)]	10 (6–27) [6/7 (86)]	1.0
	Gestation (wks), median (IQR)	37 (37–38)	37 (29–38)	0.31
	Seasonality	0 (0)	4 (4)	0.15
Clinical	Jan-March	2 (29)	7 (7)	
	April-June	2 (29)	60 (58)	
	July-September	3 (43)	33 (32)	
	October-December			
	Cases presenting in the latter half of the year, n (%)	5 (71)	91 (88)	0.24
	ICU admission, n (%)	4 (57)	12/99 (12)	0.010
	Seizures, n (%)	4 (57)	5 (5)	<
	Any	2 (29)	3 (3)	0.001
Clinical	In first 72 hours	3 (43)	5 (5)	0.03
	During admission	1 (16)	0 (0)	0.008
	Onset after discharge	3/6 ^B	1 ^B (1)	0.06
	Long-term seizures requiring ongoing therapy after discharge ^A	(50)		< 0.001
	Extra-CNS disease, n (%)	5 (71)	8 (8)	<
	Multisystemic infection ^A	4 (57)	6 (6)	0.001
	Excluding skin as the only extra-CNS involvement			0.001
	Neonates with seizures or extra-CNS disease, n (%)	6/7 (86)	10/72 (14)	< 0.001
Clinical	Coinfection with bacteria or fungus, n (%)	1 ^C (14%)	4 ^D (4)	0.28

Characteristics		HSV N = 7	Non- HSV N = 104	P- Value ^H
Imaging	Abnormal head imaging ^E , n (%)	4/7 (57)	5/26 (19)	0.068
	Presumed encephalitis, n (%) (Seizures or abnormal imaging)	6/7 (86)	7 (7) ^F	< 0.001
Hospital Stay	Length of stay (d), median (IQR)	25 (21–43)	3 (3–5)	< 0.001
Follow-Up	Follow-up (mo), median (IQR)	16 (10–24)	6 (1–12)	0.03
Mortality	Death, n (%)	1 ^G (14)	1 (1)	0.12
Neurodevelopment	Neurodevelopmental abnormalities at discharge or follow-up, n (%)	3/6 (50)	7/103 (7)	0.010
	Neurodevelopmental abnormalities in surviving infants with presumed encephalitis, n (%)	3/5 (60%)	4 /7 (57%)	1.00
Outcomes	Death or neurodevelopmental abnormalities, n (%)	4/7 (57)	8 (8)	0.002
	Overall Infants with encephalitis	4/6 (67)	4/7 (57%)	1.00
Bolded p-value denotes significance.				
Legend: CSF – cerebrospinal fluid; HSV – herpes simplex virus; IQR – interquartile range				
^A These were identified as independent risk factors after controlling for age and ICU admission, respectively.				
^B All infants with long-term seizures had neurodevelopmental delay (range mild to profound).				
^C This infant had multiple blood and CSF samples positive for <i>C. albicans</i> , which developed in the last 2 weeks of therapy for disseminated HSV.				
^D Infants received antimicrobial therapy for urinary tract infections (<i>E. coli</i> (N = 2) and <i>E. faecalis</i> (N = 2); the case of group B streptococcus coinfecting with EV is not included).				
^E This comparison was limited to those abnormalities that were consistent with CNS infection.				
^F All 7 cases with abnormal imaging or seizures had EV; the single case of HPeV did not have seizures or head imaging and therefore did not satisfy our clinical/imaging definition for encephalitis.				
^G This fatal case of HSV fulfilled criteria for encephalitis.				

Characteristics	HSV N = 7	Non- HSV N = 104	P- Value ^H
^H For comparison of proportions, Fishers exact test (2-sided) was used; for comparison of medians, Mann-Whitney test was used.			

Maternal History

Among HSV cases, three (38%) were born to mothers with active genital lesions documented at or within 7 days of delivery (Table 1). One of the three mothers had recurrent HSV1 disease and was not compliant with acyclovir prophylaxis; her infant presented with HSV1 on day 4 of life after vaginal delivery. The other two mothers had first clinical episode of genital HSV within 7 days of delivery. Their infants received no screening or empiric treatment and presented with HSV2 on days 6 and 9 after caesarian and vaginal delivery, respectively; the duration of rupture of membranes was not available. For EV infection, 3 mothers had documented illness compatible with EV within 10 days prior to delivery; their infants presented on days 5, 6 and 8. For the single case of HPeV meningitis, there was no history of preceding maternal illness.

Timing of Presentation: Early-onset infection occurred in 10 of the 111 infants (8 EV and 2 HSV). The EV cases all presented after day 2 of life and 3 of the 8 had severe disease: i) fatal myocarditis, ii) shock with coagulopathy and iii) encephalitis. The two early-onset cases with HSV infection presented on days 4 and 6.

For late-onset disease, there were 63 cases of EV meningitis, 5 cases of HSV encephalitis and one case of HPeV meningitis. All infants (N = 32) with very late onset infection had EV. All HSV cases presented before 21 days of age.

Clinical Features

There were 9 (8%) cases with seizures. Eight had seizures during the admission for the CNS infection (5 with EV and 3 with HSV) and the ninth developed seizures after discharge coinciding with CNS HSV relapse. For the 4 HSV cases, 2 had seizures only within the first 72 hours following diagnosis, 1 after 72 hours but prior to hospital discharge and 1 after discharge. One of these infants died and one with a recurrence of HSV CNS infection developed infantile spasms. Three (60%) of the 5 HSV survivors who had follow-up (8–35 months) had long-term seizures, including one who developed seizures at HSV relapse. Five of 103 infants with EV had seizures (5%), with 3 presenting in the first 72 hours following diagnosis and 2 presenting after 72 hours but prior to hospital discharge. The age at diagnosis of CNS infection in these 5 cases was 5, 10, 10, 14 and 84 days.

There were 14 (12%) infants with extra-CNS involvement. Five infants had extra-CNS HSV infection, consisting of vesicular lesions without other extra-CNS involvement (N = 1), transaminitis and pneumonitis (N = 1), transaminitis and vesicles (N = 1) and transaminitis, pneumonitis and coagulopathy

(N = 2). Coagulopathy was complicated by spontaneous intracranial haemorrhages (intraventricular and parenchymal) in one of these two infants. Extra-CNS manifestations in EV cases included rash (N = 2), pneumonia (N = 2), shock with coagulopathy (N = 2), myocarditis (N = 2) and transaminitis (N = 1). The median age of onset of the 7 cases with organ involvement (omitting the 2 with skin involvement) was 9 days (range 5–73 days). The single HPeV case was not documented to have seizures or extra-CNS disease.

Microbiology

All cases were diagnosed using PCR analysis of CSF. HSV PCR testing was also positive on skin lesions in two infants and from the conjunctiva of one (in the absence of ophthalmological abnormalities). EV and HPeV typing was not available. Suspected urinary tract coinfections occurred in 4 infants with EV infection (Table 1). Systemic candidiasis complicated the course of one infant with HSV encephalitis with liver failure, coagulopathy and intraventricular haemorrhages requiring external ventricular drain placement. *Candida albicans* was isolated from blood and CSF. Newborn screen, immunoglobulin assay and flow cytometry failed to identify an underlying immunodeficiency in this fatal case.

CSF Findings

The median values for cell count, glucose and protein on the initial CSF were not significantly different between HSV and non-HSV (Table 2). Thirty-seven (33%) infants (4 (57%) with HSV, 32 (33%) with EV and the single HPeV) had CSF white blood cell (WBC) counts less than $30 \times 10^6/\text{L}$. Notably, 5 (5%) of infants with EV infections had CSF WBC $> 2000 \times 10^6/\text{L}$.

Table 2

Comparison of initial cerebrospinal fluid findings in infants with HSV and non-HSV viral central nervous system infections by univariate analysis

Parameter	Cerebrospinal (CSF) Findings	HSV N = 7	Non-HSV N = 101 ^A	P- Value ^B
CSF White Blood Cell Count	CSF white blood cell (WBC) ($\times 10^6/L$) at diagnosis, median (IQR)	26 (2-146)	150 (14–422)	0.09
	CSF WBC, n (%)	4 (57)	33 (33) ^C	0.27
	<30 $\times 10^6/L$	3 (43)	22 (22)	0.19
	<10 $\times 10^6/L$	3 (43)	17 (17)	0.12
	<5 $\times 10^6/L$	0 (0)	10 (10)	1.00
	>1000 $\times 10^6/L$	0 (0)	6(6) ^D	
	>2000 $\times 10^6/L$			
	CSF lymphocyte percent, median (IQR)	43 (10–56)	20 (10–40)	0.26
	CSF pleocytosis ^E	3 (43)	76 (75)	0.08
	CSF cell count with polymorphonuclear dominance (> 50%), n (%)	0/7 (0)	23/86 (27)	0.19
CSF Red Blood Cell Count	CSF red blood cell count ($\times 10^6/L$), median (IQR)	26 (1-6950)	23 (2-136)	0.66
CSF Glucose	CSF glucose (mmol/L), median (IQR)	2.4 (2.1–2.8)	2.4 (2.2–2.7)	0.87
	CSF glucose < 2.1 mmol/L, n (%)	0/8 (0)	10 (10)	1.00
CSF Protein	CSF protein (g/L), median (IQR)	0.76 (0.53–0.90)	0.79 (0.56–1.02)	0.86
	Protein > 1 g/L, n (%)	1 (14)	25/99 (25)	0.68
Other	≥ 1 CSF parameter suggesting bacterial meningitis ^C , n (%)	1 (13)	65 (64)	0.006
Bolded p-value denotes significance				
Legend: CSF – cerebrospinal fluid; HSV – herpes simplex virus; IQR – interquartile range; WBC – white blood cell count				
^A Three of the non-HSV cases had CSF sent only for microbiological analysis; so only 101 cases had CSF analysis that included a cell count, protein or glucose level; 2/7 (29%) of the EV cases that were presumed to have encephalitis had CSF WBC < 30 $\times 10^6/L$.				

Parameter	Cerebrospinal (CSF) Findings	HSV N = 7	Non-HSV N = 101 ^A	P- Value ^B
^B For comparison of proportions, Fishers exact test (2-sided) was used; for comparison of medians, Mann-Whitney test was used.				
^C Parameters used were cell count > 1000 × 10 ⁶ /L, Glucose < 2.0 mmol/L and CSF Protein > 1.0 g/L.				
^D EV cases with CSF WBC > 2000 × 10 ⁶ /L had median CSF WBC of 2630 (range 2020–6400) × 10 ⁶ /L				
^E CSF pleocytosis was defined as CSF white cell count > 25 × 10 ⁶ /L for neonates and > 7 × 10 ⁶ /L for infants beyond neonatal period				

Head Imaging

Thirty-three (30%) of the 111 infants had head imaging performed (HSV (N = 7) and EV (N = 26)). Among the cases of HSV, magnetic resonance imaging was abnormal in 4/6 (67%) and appeared consistent with infection; the seventh case had only a head ultrasound which was normal. Among the cases of EV, 7/26 (27%) had abnormalities detected on imaging but only 5 (19%) of these were attributed to infection (diffusion restriction abnormalities).

Encephalitis

Thirteen infants (HSV = 6; EV = 7) fulfilled criteria for encephalitis. The EV cases presented at a median of 10 days of age (range 5 to 84 days). One case of disseminated HSV2 infection did not meet our definition of encephalitis as the infant did not have documented seizures and only had a normal head ultrasound documented, but did not have MRI or CT imaging performed. Infants with encephalitis were younger ($p = 0.012$), more likely to require ICU admission ($p < 0.001$), more likely to have disseminated disease ($p = 0.007$) and were more likely to die or have developmental delay (8 (62%) vs 4 (4%); $P < 0.001$) than those without encephalitis.

Antiviral Treatment and Prophylaxis

All HSV cases received acyclovir treatment for a median of 21 days (range 21–51 days). One infant received acyclovir until demise on day 42 of acyclovir therapy and had persistent PCR positivity in CSF. Pre-mortem CSF resistance testing returned following demise indicating that the HSV was resistant to acyclovir. Another infant did not have documented CSF clearance until 51 days of therapy. For the other 5 cases, repeat testing done between 19 and 22 days of treatment confirmed successful clearance of HSV from CSF. Three (50%) surviving infants were documented to have been discharged on oral acyclovir as prophylaxis for minimum 6 months.

Outcome

There were 2 deaths (2%), one from disseminated EV (a 6-day old infant with myocarditis who required extracorporeal membrane oxygenation) and one from HSV2 (the infant with persistent HSV detection in

CSF until death at day 48 of life). Autopsies were not performed on either infant. Virologically-proven recurrence of HSV1 encephalitis presenting as infantile spasms occurred in 1 (33%) of the 3 infants who received oral acyclovir until 6 months of life; this occurred 2 weeks after oral acyclovir was discontinued. Ten (9%) of the 109 surviving infants had neurodevelopmental sequelae documented at discharge or follow-up; 3/6 (50%) HSV survivors followed for median of 16 (8–35) months and 7/103 (7%) non-HSV survivors followed for median 6 (1–30) months ($p = 0.010$) (Table 1). Neurodevelopmental outcomes were not available for infants who had HSV persistence documented in CSF as the single survivor was lost to follow-up. All 3 of the HSV (2 HSV2; 1 HSV) and 1 of the EV survivors with neurodevelopmental sequelae developed seizure disorders requiring anticonvulsant therapy. Overall, unfavorable outcome occurred in 12 cases (11% of all viral CNS infections) but was more likely following HSV than non-HSV infection (4 (57%) versus 8 (8%); $p = 0.002$) (Table 1). Three (75%) of four HSV cases with unfavorable outcome were caused by HSV2. One of 3 (33%) cases of HSV1 had poor outcome compared to 3 of 4 (75%) cases with HSV2. All cases of EV encephalitis survived, but 4 of 7 had neurodevelopmental sequelae as compared to 3 of the 5 HSV survivors who fulfilled criteria for encephalitis (4 (57%) versus 3 (60%); $p = 1.0$). Eight (62%) of 13 infants (HSV = 4; EV = 4) with encephalitis had unfavorable outcome.

Factors associated with HSV aetiology

In univariate analysis, HSV cases were more likely to require intensive care unit (ICU) admission ($p = 0.010$), present with seizures ($p = 0.03$), have extra-CNS disease ($p < 0.001$) and have unfavorable outcome ($p < 0.001$). Seizures ($p = 0.005$) and extra-CNS disease ($p = 0.002$) remained significant after controlling for ICU admission. Among infants < 30 days of age ($N = 79$), the presence of seizures or extra-CNS disease was more likely in HSV than in non-HSV CNS infection (6 (86%) vs. 10 (14%); $p < 0.001$).

Factors associated with unfavorable outcome

In the univariate analysis, the factors associated with unfavorable outcome included younger age ($p = 0.003$), seizures ($p < 0.001$), ICU admission ($p < 0.001$), a low CSF glucose ($p = 0.03$), encephalitis ($p < 0.001$), HSV etiology ($p = 0.002$), abnormal imaging ($p = 0.017$), and extra-CNS disease ($p = 0.005$) (Table 3). In addition to these six factors, CSF protein > 1 g/L ($p = 0.02$) and ≥ 1 CSF parameter more in keeping with bacterial meningitis (cell count $> 1000 \times 10^6$ /L, Glucose < 2.0 mmol/L and CSF Protein > 1.0 g/L) were associated with unfavorable outcome in infants with EV infections ($p = 0.048$) (Table 3 footnote; Table 4). Sample size limited multivariate analysis.

Table 3

Demographic, clinical and laboratory factors associated with unfavorable outcome following viral CNS infection by univariate analysis

Factors	Unfavorable Outcome N = 12	Favorable outcome N = 99	P-Value ^B
Female Gender, n (%)	9/12 (75)	45/99 (45)	0.07
^A Age at Onset (d), median (IQR)	9 (5.5–18.5)	25 (14–33)	0.003
^A History of Seizure at Onset or During Treatment, n (%)	7/12 (58)	2/99 (2)	< 0.001
Abnormal Imaging, n (%)	7/11 (64)	4/22 (18)	0.02
^A Clinical/Imaging Criteria Fulfilled for Encephalitis, n (%)	8/12 (67)	6/99 (6)	< 0.001
^A Intensive Care Unit Admission, n (%)	7/11 (64)	9/95 (9)	< 0.001
Underlying Virus, n (%)	4/12 (33)	3/99 (3)	0.002
HSV	1/12 (8)	2/99 (2)	0.29
HSV1	3/12 (25)	1/99 (1)	0.004
HSV2			
CSF White Blood Cell Count ($\times 10^6/L$), median (IQR)	104 (27.5–762)	147 (11–358)	0.75
CSF Protein (g/L), median (IQR)	1.0 (0.64–1.27)	0.74 (0.56–0.95)	0.12
^A CSF Protein above 1 g/L, n (%)	6/12 (50)	20/94 (21)	0.07
^A CSF Glucose (mmol/L), median (IQR)	2.1 (2.0–2.45)	2.4 (2.2–2.75)	0.03

Bolded p-value denotes significance.

Legend: CSF – cerebrospinal fluid; HSV – herpes simplex virus

^AFactors that were found to be associated with unfavorable outcome in EV CNS infection. These were younger age ($p = 0.022$), seizures ($p = 0.003$), encephalitis ($p < 0.001$), ICU admission ($p = 0.004$), CSF protein > 1 g/L ($p = 0.024$), lower median CSF glucose ($p = 0.013$), ≥ 1 CSF parameter suggesting bacterial meningitis ($p = 0.048$).

^BFor comparison of proportions, Fishers exact test (2-sided) was used; for comparison of medians, Mann-Whitney test was used.

^CParameters used were cell count $> 1000 \times 10^6/L$, Glucose < 2.0 mmol/L and CSF Protein > 1.0 g/L.

Factors	Unfavorable Outcome N = 12	Favorable outcome N = 99	P-Value ^B
^A ≥1 CSF Parameter Suggestive of Bacterial Meningitis ^C , n (%)	9/12 (75)	57 /96 (59)	0.36
Extra-Central Nervous System Disease, n (%)	5/12 (42)	8/99 (8)	0.005
Bolded p-value denotes significance.			
Legend: CSF – cerebrospinal fluid; HSV – herpes simplex virus			
^A Factors that were found to be associated with unfavorable outcome in EV CNS infection. These were younger age (p = 0.022), seizures (p = 0.003), encephalitis (p < 0.001), ICU admission (p = 0.004), CSF protein > 1 g/L (p = 0.024), lower median CSF glucose (p = 0.013), ≥ 1 CSF parameter suggesting bacterial meningitis (p = 0.048).			
^B For comparison of proportions, Fishers exact test (2-sided) was used; for comparison of medians, Mann-Whitney test was used.			
^C Parameters used were cell count > 1000 × 10 ⁶ /L, Glucose < 2.0 mmol/L and CSF Protein > 1.0 g/L.			

Table 4

Demographic Clinical and Laboratory Factors Associated with Unfavorable Outcome Following Enteroviral CNS Infection

Factors	Unfavorable Outcome N = 8	Good outcome N = 95	P-Value ^A
Female Gender, n (%)	6/8 (75)	43/96 (46)	0.15
Age at Onset (d), median (IQR)	9 (5.5–19.5)	25 (14–34)	0.02
History of Seizure at Onset or During Treatment, n (%)	3/8 (38)	2/95 (2)	0.003
Abnormal Imaging, n (%)	3/7 (43)	4/19 (21)	0.34
Intensive Care Unit Admission, n (%)	4/7 (57)	8/91 (9)	0.004
CSF White Blood Cell Count $\times 10^6/L$, median (IQR)	180 (41-1271)	153 (15.5-393.5)	0.36
CSF Protein (g/L), median (IQR)	1.10 (0.73–1.27)	0.76 (0.59–0.99)	0.13
CSF Protein Over 1 g/L, n (%)	5/8 (63)	20/90 (22)	0.02
CSF Glucose (mmol/L), median (IQR)	2.1 (1.95–2.29)	2.45 (2.20–2.75)	0.01
At Least 1 CSF Parameter Suggestive of Bacterial Meningitis ^B , n (%)	8/8 (100)	57/92 (62)	0.05
Extra-CNS Disease, n (%)	2/8 (25)	6/95(6)	0.12
Bolded p-value denotes significance			
Legend: CNS – central nervous system; CSF – cerebrospinal fluid			
^A For comparison of proportions, Fishers exact test (2-sided) was used; for comparison of medians, Mann-Whitney test was used.			
^B Parameters used were cell count $> 1000 \times 10^6/L$, Glucose < 2.0 mmol/L and CSF Protein > 1.0 g/L			
Appendix 1 - ICD10CA codes used to identify potential cases			
A170 Tuberculous meningitis			
A203 Plague meningitis			
A321 Listerial meningitis and meningoencephalitis			
A390 Meningococcal meningitis			

Factors	Unfavorable Outcome N = 8	Good outcome N = 95	P- Value ^A
A870 Enteroviral meningitis			
A871 Adenoviral meningitis			
A872 Lymphocytic choriomeningitis			
A878 Other viral meningitis			
A879 Viral meningitis, unspecified			
B003 Herpesviral meningitis			
B010 Varicella meningitis			
B021 Zoster meningitis			
B051 Measles complicated by meningitis			
B261 Mumps meningitis			
B375 Candidal meningitis			
B384 Coccidioidomycosis meningitis			
G000 Haemophilus meningitis			
G001 Pneumococcal meningitis			
G002 Streptococcal meningitis			
G003 Staphylococcal meningitis			
G008 Other bacterial meningitis			
G009 Bacterial meningitis, unspecified G00 Bacterial meningitis, not elsewhere classified			
G01 Meningitis in bacterial diseases classified elsewhere			
G020* Meningitis in viral diseases classified elsewhere			
G021* Meningitis in mycoses			
G028* Meningitis in other specified infectious and parasitic diseases classified elsewhere			
G030 Nonpyogenic meningitis			
G031 Chronic meningitis			
G032 Benign recurrent meningitis [Mollaret]			

Factors	Unfavorable Outcome N = 8	Good outcome N = 95	P- Value ^A
G038 Meningitis due to other specified causes			
G039 Meningitis, unspecified			
A811 Subacute sclerosing panencephalitis			
A830 Japanese encephalitis			
A831 Western equine encephalitis			
A832 Eastern equine encephalitis			
A833 St Louis encephalitis			
A834 Australian encephalitis			
A835 California encephalitis			
A838 Other mosquito-borne viral encephalitis			
A839 Mosquito-borne viral encephalitis, unspecified			
A840 Far Eastern tick-borne encephalitis [Russian spring-summer encephalitis]			
A841 Central European tick-borne encephalitis			
A848 Other tick-borne viral encephalitis			
A849 Tick-borne viral encephalitis, unspecified			
A850 Enteroviral encephalitis			
A851 Adenoviral encephalitis			
A852 Arthropod-borne viral encephalitis, unspecified			
A858 Other specified viral encephalitis			
A86 Unspecified viral encephalitis			
A922 Venezuelan equine fever			
A923 West Nile virus infection			
B004 Herpesviral encephalitis			
B011 Varicella encephalitis			
B020 Zoster encephalitis			

Factors	Unfavorable Outcome N = 8	Good outcome N = 95	P- Value^A
B050 Measles complicated by encephalitis			
B262 Mumps encephalitis			
B582 Toxoplasma meningoencephalitis			
G040 Acute disseminated encephalitis			
G042 Bacterial meningoencephalitis and meningomyelitis, not elsewhere classified			
G048 Other encephalitis, myelitis and encephalomyelitis			
G049 Encephalitis, myelitis and encephalomyelitis, unspecified			
G050* Encephalitis, myelitis and encephalomyelitis in bacterial diseases classified elsewhere			
G05.1* Encephalitis, myelitis and encephalomyelitis in viral diseases classified elsewhere			
G052* Encephalitis, myelitis and encephalomyelitis in other infectious and parasitic diseases classified elsewhere			
G058* Encephalitis, myelitis and encephalomyelitis in other diseases classified elsewhere			

Discussion

Viral infections accounted for about two-thirds of CNS infections in the first 90 days of life where a CSF pathogen was detected in the current study. Trends in Canada are not clear but in a population-based United Kingdom (UK) study, the authors document a dramatic rise in admissions for viral meningitis in infants between 2005 and 2011.⁶ They show a major increase in the proportion of cases of viral meningitis recognized to be due to EV over time, from 90 (3%) of 2770 admissions for viral meningitis between 1968 and 1985 to 811 (47%) of 1716 viral meningitis admissions in 2007-2011.⁶ These changing trends may reflect the UK adoption of molecular diagnostic screening for viral meningitis resulting in increased detection over conventional viral culture methods which were not consistently applied in earlier years.⁷ Further, molecular testing has facilitated the detection of viruses like HPeV3 that will be missed by viral isolation techniques.⁷

Consistent with prior literature, in the current study HSV was much more likely than EV to lead to encephalitis, long-term neurodevelopmental morbidity or death.^{3,8-11} However, among the subgroup of EV cases with encephalitis, we observed a similar rate of adverse outcome to that with HSV encephalitis. Identifying clinical or laboratory markers that distinguish HSV from non-HSV viral infections is vital to ensure that empiric acyclovir is started at presentation in all HSV cases.⁴ We identified younger age, seizures, ICU admission and the presence of extra-CNS features as factors associated with HSV infection;

however, only seizures and extra-CNS disease remained significant in the multivariate analysis and 3 of 7 infants with HSV CNS disease did not have seizures. Most if not all HSV encephalitis in the neonatal period comes from perinatal transmission, and in our study all presented by day 21 of life. However, HSV encephalitis can present at any age and in a recent study of 46 cases up to 60 days of age, the IQR was 9 to 24 days.¹²

Most genital HSV infections are subclinical. A small percentage of neonatal HSV cases may arise from post-natal transmission from saliva.⁸ Therefore, all infants should be assumed to be at risk of HSV infection irrespective of maternal history. In addition, as demonstrated in 4 of the 7 infants with CNS HSV in our cohort, the absence of CSF pleocytosis does not exclude CNS HSV infection. Furthermore, one case in our cohort had HSV detected by PCR on CSF analysis from day 5 of illness after a negative PCR on day 2 of illness. Thus, if the clinical picture is suggestive of HSV infection and initial CSF HSV testing returns negative, acyclovir should be continued until another CSF sample can be retested to ensure that the original sample was not falsely negative.¹³ The importance of performing CSF analysis towards the end of treatment course is exemplified by the two cases with persistent detection of HSV in CSF, which led to continuing intravenous acyclovir beyond the usual 21-day course.

As noted in our fatal HSV2 case, the possibility of acyclovir resistance should be considered in children with persistent detection of the virus in CSF¹⁴⁻¹⁸. While rare, the possibility of acyclovir resistance needs to be kept in mind as alternative therapy including foscarnet or vidarabine may be of benefit.¹⁴⁻¹⁶

Limitations of our study include the retrospective design, the lack of EV and HPeV typing and the lack of HPeV testing at most study sites. It is likely that other viral etiologies of meningitis or encephalitis will eventually be identified. Methods of molecular testing varied by study center. Emerging molecular diagnostic panels may eventually improve diagnosis of CNS infections (<https://www.biofiredx.com/products/the-filmarray-panels/filmarrayme/>). Infants with mild viral meningitis are not always recognized to have CNS infection. The total number of infants investigated at the 7 centers to yield the 174 with proven CNS infections is not known. Application of the International Encephalitis Consortium definition of encephalitis¹⁹ to infants may be inaccurate as it can be difficult to determine if they have altered level of consciousness or focal signs and they are less likely than older children to have fever or CSF pleocytosis. An EEG is not always performed. Therefore, we used a simplified definition for encephalitis which was highly dependent upon the decision to perform and the interpretation of head imaging (which was not always obtained) and recognition of seizures so could have missed or over-diagnosed cases. Infants who had coagulopathy or were too systemically ill to have CSF obtained or who died before they had a diagnosis would have been missed. Molecular testing for HSV (and presumably for other viruses) can be falsely negative early in the course of infection. However, the inclusion of only proven cases was deemed to yield the most accurate data. There was inconsistent access to data on neurodevelopment follow-up and this follow-up was not standardized between centers.

Conclusions

Viral CNS infections appear to be more common than bacterial infections in the first 90 days of life. Age < 21 days and presence of seizures or extra-CNS involvement are clues to HSV infection, even in the absence of CSF pleocytosis. However, not all infants with CNS HSV have seizures. Although most infants with non-HSV viral infections have good outcomes, the subset who have seizures and/or abnormal head imaging may have outcomes similar to those of infants with HSV encephalitis and require neurodevelopmental follow-up. Further studies should address the contribution of HPeV to viral CNS infections and explore predictors of long-term morbidity.

Declarations

Ethics approval and consent to participate: Ethics approval was obtained at each site for conduct of this study with the primary approval coming from the Health Research Ethics Board of the University of Alberta (Study number PRO00055909). Parental consent was waived as it was a retrospective chart review.

Consent for publication: Not applicable

Availability of data and material: All data are stored in REDCap. An anonymized version is available from the corresponding author upon reasonable requests.

Competing interests: Joseph Ting is an Associate Editor for *BMC Pediatrics*.

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Authors' contributions: JR and MB wrote the first draft of the protocol, designed the case report form and finalized the manuscript. MB performed the data analysis. DP wrote the first draft of the manuscript. CR, LO, JB1, JB2, SK, AB, JM, AB, JT and AR provided input into the protocol, case report form or manuscript and organized data collection. All authors approved the final version.

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Abbreviations

CNS – central nervous system

CSF – cerebrospinal fluid

EV – enterovirus

HSV – herpes simplex virus

IQR – interquartile range

WBC – white blood cell count

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