

Enterovirus-Associated Encephalitis in the California Encephalitis Project, 1998–2005

Ashley L. Fowlkes,¹ Somayeh Honarmand,² Carol Glaser,² Shigeo Yagi,² David Schnurr,² M. Steven Oberste,¹ Larry Anderson,¹ Mark A. Pallansch,¹ and Nino Khetsuriani¹

¹Centers for Disease Control and Prevention, Atlanta, Georgia; ²California Department of Public Health, Richmond

Background. Encephalitis is a relatively rare presentation of enterovirus (EV) infections. Clinical and epidemiologic characteristics of EV encephalitis (EVE) have not been well characterized.

Methods. Patients with encephalitis enrolled in the California Encephalitis Project from 1998 to 2005 were tested for a range of pathogens, including EV, using a standardized diagnostic algorithm. EVE was categorized as “confirmed” (EV detected in cerebrospinal fluid [CSF] or brain tissue) or “possible” (EV found in respiratory or fecal specimens or serum EV immunoglobulin [Ig] M detected). We compared clinical and epidemiologic characteristics of EVE with those of other infectious encephalitis cases.

Results. EVE was diagnosed in 73 (4.6%) of 1571 patients (45 confirmed cases, 28 possible cases); 11.1% of cases had other infectious causes. Patients with confirmed EVE were younger, although 27% were adults, who presented with significantly less severe symptoms. Serotypes identified in EVE cases correlated with the predominant serotype for the given year reported to the National Enterovirus Surveillance System at the Centers for Disease Control and Prevention. Two of 4 fatal EVE cases were associated with EV71.

Conclusion. EVs are an important cause of encephalitis cases requiring hospitalization, in both children and adults. Our data suggest that EVE severity varies by serotype, confirm the importance of CSF/brain tissue polymerase chain reaction, and demonstrate that serum IgM findings are of little value in diagnosing EVE.

In the United States, encephalitis causes an estimated 19,000 hospitalizations and up to 1400 deaths each year [1, 2]. Depending on the diagnostic methods used and the criteria for linking the detected agent to the disease, $\geq 60\%$ of hospitalized patients with encephalitis may have no etiologic agent identified [3]. Enteroviruses (EVs) are a primary cause of aseptic meningitis and are among the more commonly detected viral causes of encephalitis [4, 5]. Studies in the United States and various countries have reported EV in $<1\%$ to 15% of all encephalitis cases [6–11].

EVs are among the most common viruses infecting humans, estimated to be associated with 10–15 million symptomatic infections in the United States each year [12]. The genus *Enterovirus* (family Picornaviridae) includes 4 species of human EV (A, B, C, and D), with >80 currently recognized serotypes [13]. Clinical manifestations vary widely, from asymptomatic infection to mild febrile illness to more severe diseases, including meningitis, myocarditis, paralytic illness, and encephalitis. Because EV infections are common, even uncommon complications can translate into substantial disease burden. EV encephalitis (EVE) is typically a rare complication of EV infection and often presents in conjunction with a meningeal infection, resulting in a “meningoencephalitis” [14]. Sometimes EVE may also present as a focal encephalitis, suggestive of a herpes simplex encephalitis [15–17]. In neonates, a variety of EVs have also been associated with serious complications, including encephalitis and death [18, 19]. Outbreaks involving substantial numbers of EV71 encephalitis cases have been observed among young children in some countries [20, 21]. EV infections are predominantly reported among children, but adults who have not been previously exposed to a particular EV can also be infected [14].

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Reprints or correspondence: Dr. Fowlkes, 1600 Clifton Rd. NE, Mailstop A34, Atlanta, GA 30333 (ahl4@cdc.gov).

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We analyzed EVE cases identified in the California Encephalitis Project (CEP), a large study of encephalitis cases conducted by the California Department of Public Health, through the Emerging Infectious Program of the Centers for Disease Control and Prevention (CDC). These cases provided an opportunity to describe the clinical and epidemiologic features of EVE and compare these with findings in encephalitis caused by other infectious agents.

METHODS

The methods for the CEP have been described elsewhere [3]. Briefly, patients hospitalized with encephalitis during 1998–2005 were referred by physicians to the CEP. Immunocompetent patients aged ≥ 6 months who met the study case definition of encephalitis were enrolled. A case of encephalitis was defined as encephalopathy (depressed or altered level of consciousness lasting ≥ 24 hours, lethargy, or change in personality) requiring hospitalization and with ≥ 1 of the following findings: fever, seizure, focal neurologic findings, cerebrospinal fluid (CSF) pleocytosis, and electroencephalographic (EEG) or neuroimaging findings consistent with encephalitis. Clinical and epidemiologic data were obtained from the referring physician. Biological specimens collected for routine diagnostic testing, including brain tissue, CSF, acute- and convalescent-stage serum, respiratory swab, stool, or autopsy specimens, were submitted for standardized testing to the California Viral and Rickettsial Disease Laboratory. As described elsewhere [3], specimens were tested for a core set of pathogens associated with encephalitis, including EV. Additional tests were performed selectively, depending on exposure information, clinical presentation, and specimen availability.

Laboratory testing for EV included pan-EV reverse-transcriptase polymerase chain reaction (PCR), viral culture, and EV IgM antibody detection. The serotype was determined by indirect immunofluorescence using monoclonal antibodies, by neutralization using polyclonal antibodies, or by sequencing of the VP1 gene [22]. Encephalitis cases with EV detected in CSF or brain tissue were categorized as confirmed EVE. Cases with other evidence of EV infection, including EV detected by PCR or culture only in non-central nervous system (CNS) specimens (respiratory or fecal specimens) or by the presence of serum EV IgM, were categorized as possible EVE. The 4 diagnostic groups—confirmed EVE, possible EVE, encephalitis with other confirmed or probable viral causes, and encephalitis with confirmed or probable nonviral causes—were used for analysis. Cases with Creutzfeldt-Jakob disease were excluded from analysis. We also compared possible EVE cases with diagnosis based on positive viral culture or PCR detection of EV from nonsterile sites [EV PCR/culture positive] with possible EVE cases with diagnosis based solely on detection of IgM antibodies against EV in an acute serum specimen [EV IgM positive]. Confirmed and

probable categories for non-EV cases were defined by pathogen-specific algorithms, described elsewhere [3].

Serotypes associated with EVE cases in the study were compared with predominant serotypes circulating in California and nationwide for the corresponding year and reported to the National Enterovirus Surveillance System (NESS), CDC [23]. Data were analyzed using SAS software (version 9.0; SAS Institute). We compared characteristics of confirmed EVE cases with those of possible EVE and other infectious encephalitis cases. We compared proportions using the Mantel-Haenszel χ^2 test or Fischer's exact test when necessary. Analysis of variance was used to determine whether there were significant differences in age groups between etiologic categories, and a nonparametric Wilcoxon rank sum test was used to compare findings by age and length of hospitalization as continuous variables.

RESULTS

EVs were detected in 73 patients with of encephalitis, accounting for 4.6% of all 1571 patients enrolled in the CEP during 1998–2005. Of these, 45 patients met the definition of confirmed EVE (2.9% of total enrollment, 20.5% of 220 patients with a confirmed infectious cause, and 26.2% of 172 patients with a confirmed viral cause). The other 28 EV-positive patients (1.8% of total enrollment) were classified as having possible EVE, with 14 positive by EV IgM antibody enzyme immunoassay and 14 positive by EV PCR or tissue culture isolation from a non-CNS specimen. Confirmed and probable infectious causes were viruses other than EV in 127 cases (8.1% of all cases) and nonviral (bacterial, fungal, or parasitic) in 48 cases (3.1%).

Demographics. Table 1 illustrates the age, sex, and race distribution of patients with encephalitis by comparison group. EVE occurred across a wide age range (6 months to 74 years), with no significant differences between patients with confirmed and those with possible EVE. However, both of these groups were significantly younger (median ages, 12 and 15 years, respectively) than patients with encephalitis due to other viral infections (median age, 44 years; $P < .01$ for both comparisons). Although the majority of confirmed EVE cases occurred among children, 27% occurred among adults aged ≥ 18 years. Adults aged ≥ 18 years accounted for 43%–46% of encephalitis cases in the other diagnostic categories.

Patients with EVE were predominantly male, similar to the breakdown in the other etiologic categories. The racial distribution of patients with confirmed and possible EVE tended to reflect the California population (46% white, 7% black, 30% Hispanic, 17% other races [5]) (table 1). The only significant differences among comparison groups were a lower proportion of white subjects among patients with confirmed EVE than among those with other viral causes (36% vs. 56%; $P < .05$) and a higher proportion of Hispanics among patients with possible EVE than among those with other viral causes.

Table 1. Demographic, clinical, and laboratory findings among patients with encephalitis with infectious causes.

Finding	Patients, no. (%)				P		
	Confirmed EVE (n = 45)	Possible EVE (n = 28)	Other viral causes (n = 127)	Other nonviral causes (n = 48)	Confirmed vs. possible EVE	Confirmed EVE vs. other viral cause	Confirmed EVE vs. nonviral cause
Demographic characteristic							
Female sex	20 (44.4)	11 (39.3)	58 (45.7)	16 (33.3)	NS	NS	NS
Age, years							
<5	12 (28.9)	8 (28.6)	11 (8.66)	8 (16.7)	NS	<.001	NS
5–9	6 (13.3)	3 (10.7)	9 (7.09)	9 (18.8)			
10–19	14 (31.1)	5 (17.9)	23 (18.2)	10 (20.8)			
20–50	9 (20)	8 (28.6)	27 (21.3)	9 (18.8)			
≥50	3 (6.7)	4 (14.3)	57 (44.9)	12 (25)			
Race							
White	15 (35.7)	11 (44)	61 (56.5)	16 (33.3)	NS	<.05	.06
Black	4 (9.5)	2 (8.0)	7 (6.5)	3 (6.3)	NS	NS	NS
Hispanic	14 (33.3)	11 (44)	24 (22.2)	21 (43.8)	NS	NS	NS
Other	9 (21.4)	1 (4)	16 (14.8)	8 (16.7)	.05	NS	NS
Clinical symptom							
Altered consciousness	19 (46.3)	17 (63)	86 (68.8)	36 (75)	NS	<.05	<.01
Lethargy	29 (64.4)	16 (61.5)	100 (80)	46 (97.9)	NS	<.05	<.001
Personality change	5 (11.9)	12 (46.2)	52 (41.9)	22 (48.9)	<.01	<.001	<.001
Hallucination	2 (4.8)	0 (0)	16 (13.9)	9 (20)	NS	NS	<.05
Stiff neck	19 (43.2)	11 (42.3)	34 (28.1)	24 (52.2)	NS	.08	NS
Ataxia	14 (35)	9 (50)	28 (28.3)	15 (34.9)	NS	NS	NS
Seizure	13 (28.9)	9 (32.1)	51 (41.5)	21 (43.8)	NS	NS	NS
Coma	2 (4.6)	5 (17.9)	21 (16.9)	15 (31.9)	.06	<.05	<.001
Somnolence	15 (34.9)	2 (28.6)	49 (40.5)	29 (63)	NS	NS	<.01
Focal neurologic findings	12 (27.3)	3 (60)	43 (36.1)	19 (42.2)	NS	NS	NS
Laboratory findings^a							
CSF pleocytosis	32 (71.1)	22 (78.6)	101 (79.5)	34 (70.8)	NS	NS	NS
Predominance of lymphocytes in CSF	9 (33.3)	8 (57.1)	50 (66.7)	15 (53.6)	NS	<.01	NS
Elevated CSF proteins	21 (48.8)	16 (61.5)	79 (65.8)	33 (71.7)	NS	.05	<.05
Imaging and EEG findings							
Abnormal MR imaging findings	10 (47.6)	9 (52.9)	63 (70.8)	23 (69.7)	NS	.07	NS
Abnormal EEG findings	5 (55.6)	8 (66.7)	55 (88.7)	22 (71)	NS	<.05	NS

NOTE. Data are from the California Encephalitis Project, 1998–2005. Denominators used to calculate percentages varied depending on the available data. CSF, cerebrospinal fluid; EEG, electroencephalographic; EVE, enterovirus encephalitis; MR, magnetic resonance; NS, not significant.

^a CSF pleocytosis was defined as ≥5 white blood cells/mm³; lymphocyte predominance, >60% lymphocytes; and elevated CSF protein, levels >45 mg/dL.

Temporal trends. The distribution of EVE cases by year of onset is given in table 2. The proportion of EVE among all referred cases varied over time between a low of 2.5% in 2004 and a high of 7.4% in 2003. Two years—2003 and 2005—accounted for most confirmed EVE cases (26.7% and 28.9% of all confirmed EVE cases, respectively). EVE cases were reported throughout the year, but most of them occurred during the 5-month period of June–October (64% for confirmed EVE, 61% for possible EVE) (figure 1). The summer–fall peak was also observed for the group with other viral causes of encephalitis (56% during June–October) but not for the group with nonviral causes (48%) (figure 1).

Clinical course and presentations. Table 1 shows clinical findings and laboratory test results by study group. Overall, patients with EVE appeared to have less severe clinical presentations than patients in other etiologic groups. Patients with confirmed EVE had lower frequencies of coma, altered consciousness, lethargy, and personality change than patients with other viral or nonviral causes, as well as lower frequencies of hallucinations and somnolence than patients with nonviral causes. In addition, the confirmed EVE group had a lower proportion of patients with abnormal EEG findings than the group with other viral causes of encephalitis, and lower median levels of CSF protein than the group with nonviral causes (table 2).

Table 2. Enterovirus serotypes associated with encephalitis and predominant serotypes circulating in the United States reported to the National Enterovirus Surveillance System (NESS), 1998–2005.

Year	Patients enrolled in CEP, no. (n = 1571)	Patients, no. (%)		EV serotypes associated with encephalitis cases	Most common serotypes in the United States reported to the NESS		Most common serotypes in California reported to the NESS	
		Confirmed EVE (n = 45)	Possible EVE (n = 28)		Serotype	Reported EVs, %	Serotype	Reported EVs, %
1998	38	0 (0)	2 (5.3)	Not typed	E30	54	E9	41.2
					E9	14.2	E30	29.4
					E11	7.1	CVB3	11.8
					CVB3	4.3	E11	5.9
					E6	4.1	E18	5.9
1999	133	4 (3.0)	4 (3.0)	Not typed	E11	48.1	E11	100
					E16	13.1		
					E9	9.6		
					E14	5.5		
					E25	4.8		
2000	155	2 (1.3)	7 (4.5)	CVA9, CVA21, E19	CVB5	32.9	CVB5	18.2
					E6	9.6	CVB4	13.6
					CVA9	9.1	CVA9	11.4
					CVB4	8.4	CVA21	11.4
					E11	6.8	E11	9.1
2001	148	4 (2.7)	2 (1.4)	E13, E18	E13	30.2	E13	36.8
					E18	29.3	E18	36.8
					CVB2	7.7	CVB1	5.9
					E6	5.3	CVB2	4.4
					E4	3.9	E6	4.4
2002	261	5 (1.9)	3 (1.1)	E15, E18	E7	22	E18	27.3
					E9	19.1	E9	18.2
					CVB1	9.8	E13	18.2
					E18	6.6	CVB3	9.1
					E13	6.3	CVB5	9.1
2003	204	12 (5.9)	3 (1.5)	E30, CVB4	E9	41	E30	76.2
					E30	32.4	E9	16.8
					CVB1	4.6	CVB1	1.2
					CVB4	2.9	CVB4	1.2
					CVA9	2.7	E4	1.2
2004	278	5 (1.8)	2 (0.7)	E9, E30, EV71, CVB5	E30	40.5	E9	43.6
					E9	18.9	E30	9.7
					CVB5	7.2	CVA9	8.1
					CVB4	5.3	CVB4	8.1
					CVB3	4.7	CVB3	4.8
2005	354	13 (3.7)	5 (1.4)	E6, CVB2, CVB3, CVA9	CVB5	18.9	CVB5	26.7
					E6	16.4	E6	20
					E30	15.1	E30	13.3
					E18	7.3	CVA9	13.3
					CVB3	7.2	CVB3	6.7

NOTE. CEP, California Encephalitis Project; CVA, coxsackievirus A; CVB, coxsackievirus B; E, echovirus; EV, enterovirus; EVE, enterovirus encephalitis.

Similar to patients with confirmed EVE, patients with possible EVE had a significantly lower proportion of lethargy than other infectious groups. They also tended to have a lower proportion of abnormal EEG findings ($P = .05$) than did patients with other viral causes.

When confirmed and possible EVE groups were compared with each other, the only significant difference was the lower frequency of personality change among confirmed cases. There was also a lower proportion of patients with coma among those with confirmed EVE, but the difference did not reach statistical

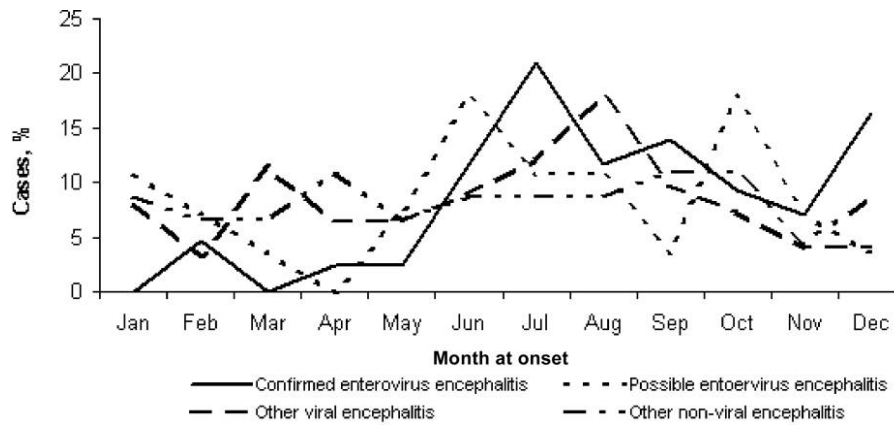


Figure 1. Seasonal distribution of encephalitis cases with infectious causes (California Encephalitis Project, 1998–2005). The month of hospital admission was used if the date of onset of encephalitis was not available.

significance ($P = .06$). The analysis of prodromal illness identified no significant differences between comparison groups with regard to upper respiratory tract symptoms, gastrointestinal symptoms, rash, or fever higher than 38°C (data not shown).

Overall, patients with EVE had a shorter median duration of hospitalization (5 days for confirmed, 8 days for possible EVE) than other etiologic groups (11 days for other viral causes, 13 days for nonviral causes), but this difference was significant only for confirmed EVE compared with other viral and nonviral groups ($P < .05$ and $P < .01$, respectively). Intensive care unit (ICU) treatment was required significantly less commonly for EVE (46.2% of cases for confirmed, 59.3% for possible EVE) than for encephalitis with nonviral causes (83.7%; $P < .001$ for confirmed, $P < .05$ for possible EVE), but not less commonly than for encephalitis with other viral causes (57.8%) (table 1). Although fatal outcome was less common for EVE (9% for confirmed EVE, no fatal cases for possible EVE) than for other groups (14% for other viral causes, 17% for nonviral causes) the differences between groups were not significant.

Serotype variation. The EV serotype was determined for 20 (27.4%) of 73 EVE cases (16 confirmed, 4 possible). Echovirus 30 accounted for 7 cases, EV71 for 3, and echovirus 18 for 2, with 1 case each for coxsackievirus A9, A21, B4, and B5 and echovirus 9, 13, 15, and 19. Of 4 fatal EVE cases, 2 were associated with EV71, and EVs were not typed in the other 2 cases. Both of these deaths (in patients aged 11 and 56 years) occurred in 2002. The elder of these patients had a newly diagnosed concurrent HIV infection. Serotypes associated with EVE varied over time and generally correlated with the predominant EVs for the given year circulating in California or nationwide, as reported to NESS at the CDC (table 2).

Examination of possible EVE cases. In a comparison of the 2 categories of possible EVE cases, the median age for the EV PCR/culture-positive group was 6.5 years, similar to that in patients with confirmed EVE. In contrast, the median age for the EV IgM-positive group was 30.5 years, much higher than among

patients with confirmed cases ($P = .07$). In a categorical comparison, children (age <18 years) accounted for a significantly lower proportion of cases in the EV IgM-positive group (29%) than in the EV PCR/culture-positive (86%) and confirmed EVE (71%) groups ($P < .01$ for both comparisons). In the analysis of the seasonal distribution of cases, the June–October peak was more prominent for the EV PCR/culture-positive group than for the EV IgM-positive group (71% vs. 50%, respectively), but the difference was not significant owing to the small numbers of cases in these groups. When severity of disease was compared, there was a trend toward ICU treatment being required more frequently for EV PCR/culture-positive patients with possible EVE than for EV IgM-positive patients with possible EVE group or patients with confirmed EVE (77% vs. 43% and 46%; $P = .07$ and $P = .05$, respectively).

DISCUSSION

This analysis of a large series of encephalitis cases supports the findings of other studies showing that EVs are among the important known causes of encephalitis, accounting for about 5% of cases in this study. This proportion may be an underestimate, for at least 2 reasons. First, cases were often referred to this study because the initial laboratory work-up failed to identify the cause. EV assays are available in many diagnostic virology laboratories; some EVE cases would have been identified in the initial round of testing and therefore missed by the study. This referral bias is a likely explanation for a lower than expected proportion of another common cause of encephalitis—herpes simplex virus—in the population referred to the CEP [5]. Second, in some cases referred to the study, the diagnosis of EVE may have been missed. EVs are usually present in the CSF only transiently; therefore, the time period when they can be detected in CSF, even by highly sensitive assays such as PCR, is brief. In addition, cases of EVE could be missed by CSF PCR if the disease process does not involve the meninges. In such cases, the virus may be

present in the brain parenchyma but not the CSF. Furthermore, in cases without meningeal involvement, the virus may be present in respiratory or stool specimens, but is more difficult to link to the disease.

The clinical and epidemiologic evaluation of possible EVE cases confirmed the limited diagnostic utility of PCR in non-CSF specimens, particularly serum IgM, by demonstrating differences between each group of possible EVE cases and the confirmed EVE cases. Detection of EVs from non-CNS sites or serologic evidence of infection, (i.e., IgM positivity) does not confirm a link to the encephalitis. Although the EV presence in the CSF may be transient, the virus can be detected in throat swab specimens for days and in stool specimens for weeks after onset [24]. However, because EVs are common viruses that often lead to asymptomatic infections, EVs detected outside the CNS could represent incidental findings not associated with an acute neurologic illness, such as encephalitis. EV IgM antibodies are similarly difficult to connect to an acute illness, because they can sometimes be detected several months after acute infection [25]. These same types of diagnostic issues confound the linking of other agents to encephalitis and our ability to diagnose the cause of encephalitis [3, 5].

Our data suggest that, overall, EVE presents as a milder clinical illness resulting in a shorter length of hospital stay than encephalitis due to other infectious causes. We observed a lower proportion of patients presenting with coma and lethargy for EVE compared with the other infectious causes. Other clinical indicators of severe disease, such as altered consciousness, personality change, hallucinations, or somnolence, were also less frequent. Our data also demonstrated that although EVE is typically perceived as a pediatric disease, adult patients are relatively common, comprising nearly one-third of patients with EVE. This finding suggests that the high index of suspicion for EVs as a potential cause of encephalitis should be maintained, irrespective of the patient's age.

Although there are many EVE serotypes, and most cause a range of similar illnesses, there are differences in the clinical and epidemiologic patterns of infection. Differences in age distribution and outcome of EV infections by serotype have also been found [19, 23, 26]. To determine whether these patterns might also apply to EVE—that is, whether some EVs were more likely to cause severe neurologic disease—we reviewed the serotypes detected in our study. Investigators at the CDC have collected information on EV detections by serotype for many years [23], and these data provide some insights into the serotypes most often associated with CNS illnesses, including EVE.

Although specific diagnoses are not reported to NESS, the source of the specimen for viral detection is included. When we considered a CSF specimen to be a likely indicator of associated neurologic illnesses, including meningitis and encephalitis, NESS data suggested that the following EVs probably had a greater propensity for causing neurologic illness than other EVs:

echoviruses 4, 5, 6, 7, 9, 11, 13, 18, and 30 and coxsackieviruses A9, B2, B4, and B5 [23]. Some of these serotypes (echoviruses 9, 13, 18, and 30; coxsackieviruses B3 and B5), as well as EV71, have been reported to cause outbreaks of aseptic meningitis, encephalitis, or poliomyelitis [27–31]. Interestingly, the highest proportion of EVE (7.4% of all patients) noted in this study was noted in 2003, the year in which 2 highly neurotropic EVs, echoviruses 9 and 30, circulated nationwide, causing widespread outbreaks of aseptic meningitis [27]. Most of the serotypes identified in patients with EVE in this study were the ones more likely to be associated with neurologic illness, according to NESS data.

The determination of causative serotypes may also have implications for clinical prognosis. Our data supported the differences by serotype in disease severity and outcome of EVE and confirmed the association of EV71 with severe illness and a high proportion of deaths. Although at present no specific antiviral treatment is available for EV infections, development is currently ongoing, and differential sensitivity of various serotypes to at least 1 candidate drug, pleconaril, has been shown [32], supporting the potential clinical utility of EV serotype determination. The introduction of a PCR assay for EV with subsequent typing by sequencing of the VP1 gene [33] has greatly enhanced EV detection methods and facilitated the ability of clinicians to identify the implicated serotype.

In summary, detection of an etiologic agent in encephalitis cases is complicated, resulting in a low proportion of cases with known causes. In this context of the overall difficulty of determining the cause of encephalitis, EVs represent an important and diagnosable cause. The results of this study demonstrate that EVs contribute to a significant proportion of encephalitis cases in all age groups, and their role can be confirmed by PCR testing of CSF or brain tissue. Patients with confirmed EVE, with the exception of EVE due to EV71, present with milder clinical illness than patients with encephalitis caused by other infections. In addition to their utility for public health surveillance purposes, EV serotype data may be helpful in predicting the outcome of encephalitis and in the future development of antiviral treatments.

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