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MAJOR ARTICLE

Clinical Characteristics of the 2019 Eastern Equine Encephalitis Outbreak in Michigan

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Background. Eastern equine encephalitis virus is a mosquito-borne alphavirus responsible for unpredictable outbreaks of severe neurologic disease in animals and humans. While most human infections are asymptomatic or clinically nonspecific, a minority of patients develops encephalitic disease, a devastating illness with a mortality rate of ≥30%. No treatments are known to be effective. Eastern equine encephalitis virus infection is rare in the United States, with an annual average nationwide incidence of 7 cases between 2009 and 2018. However, in 2019, 38 cases were confirmed nationwide, including 10 in Michigan.

Methods. Data from 8 cases identified by a regional network of physicians in southwest Michigan were abstracted from clinical records. Clinical imaging and histopathology were aggregated and reviewed.

Results. Patients were predominantly older adults (median age, 64 years), and all were male. Results of initial arboviral cerebrospinal fluid serology were frequently negative, and diagnosis was not made until a median of 24.5 days (range, 13–38 days) after presentation, despite prompt lumbar punctures in all patients. Imaging findings were dynamic and heterogeneous, with abnormalities of the thalamus and/or basal ganglia, and prominent pons and midbrain abnormalities were displayed in 1 patient. Six patients died, 1 survived the acute illness with severe neurologic sequelae, and 1 recovered with mild sequelae. A limited postmortem examination revealed diffuse meningoencephalitis, neuronophagia, and focal vascular necrosis.

Conclusions. Eastern equine encephalitis is a frequently fatal condition whose diagnosis is often delayed, and for which no effective treatments are known. Improved diagnostics are needed to facilitate patient care and encourage the development of treatments.

Keywords. Eastern equine encephalitis; arbovirus; viral encephalitis.

Eastern equine encephalitis (EEE) is a neuroinvasive infectious disease caused by infection with EEE virus (EEEV), an arbovirus of the alphavirus group. EEEV is enzootic in a variety of migratory birds [1], which are thought to annually seed northern locations of the United States from areas of year-round persistence of the virus in the southeast [2], although northern overwintering of the virus also occurs [3]. EEEV causes sporadic cases of human disease across a broad geographic range of eastern North America and the Caribbean and was first isolated from the brain of an infected horse in 1933 [4]. As its name suggests, EEEV is responsible for epizootics of fatal encephalitis in horses, which have historically been followed by human outbreaks of varying size: the 1938 outbreak involved 34 human cases with 25 deaths in Massachusetts, while the 1959 outbreak in New Jersey afflicted 32 individuals [5]. Since 1959, lower levels of human EEE activity in the United States have been more typical, with no year exceeding 15 cases between 1965 and 2018. The median number of annual human cases between 2010 and 2018 was 7 nationwide [6].

During the summer of 2019, a multistate outbreak of EEE occurred, resulting in the highest annual case count yet recorded, at 38 nationwide [7]. Notably, while the majority of cases continued to be seen in the Northeast, Michigan emerged as the state with the second-highest number of EEE diagnoses, with 10 cases clustered geographically in the southwest portion of the state [6]. The mechanisms underlying this marked year-to-year and geographic variability are likely multifactorial, involving climatic factors, especially rainfall [8], as well as ecological dynamics between the avian host reservoir, the enzootic *Culiseta melanura* mosquito vector [9], human behavior, and possible bridging vectors thought to be responsible for infecting humans and horses,

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including mosquitoes of the *Aedes*, *Coquillettidia*, and *Culex* genera [4, 10].

The tendency of rising global temperatures to be accompanied by increased rainfall in temperate locations has led to concern that climate change could lead to higher EEE disease burdens, and indeed there has been a trend toward higher annual case totals in the United States since 2003 [5, 11]. The potential for arboviruses to adapt to new mosquito vectors [12] and cause pandemic outbreaks [13] has contributed to calls for increased attention to the threat posed by EEEV on the part of public health authorities [11]. Owing to its virulence, the lack of approved human vaccines and treatments, and its potential for use as a bioweapon, EEE has been designated a select agent by the US Centers for Disease Control and Prevention (CDC) and the US Department of Agriculture.

Clinically, EEE is an ominous diagnosis, and carries the highest case fatality rate seen among North American mosquito-borne diseases, variously cited as 35% to 75% [5]. Yet encephalitis is not the typical outcome of EEEV infection: an estimated 96% of human EEEV infections are asymptomatic [11], while nonencephalitic, symptomatic EEEV infections are rarely diagnosed and therefore poorly understood. Following a bite by an infected mosquito, an incubation period of 4–10 days ensues, after which either a nonspecific febrile illness or a neuroinvasive disease manifests. Meningitis has been described, but encephalitis is the typical neuroinvasive manifestation [14]. Historically, children and older persons are most likely to be affected [4], while for the 2019 nationwide outbreak specifically, more than three-quarters of the patients were male, with a median age of 64 years [7].

Affected patients typically present after a short febrile prodrome with neurologic symptoms, especially confusion, somnolence, and seizures, which typically progresses to coma [14]. Brain imaging frequently demonstrates abnormalities in the thalamus and basal ganglia. Diagnosis of EEE is typically accomplished via detection of anti-EEEV antibodies in cerebrospinal fluid (CSF) or serum samples, performed at commercial reference and public health laboratories, with molecular detection of viral RNA available only at select public health laboratories; however, poor sensitivity of commercially available CSF serologic tests has been identified as a diagnostic concern [15]. Treatment is supportive as no specific therapy is available, although intravenous immunoglobulin (IVIG) and corticosteroids have been administered [16]. Nearly all surviving patients have residual impairment, ranging from mild deficits to 24-hour care, posing a significant economic burden [17] and highlighting the need for effective therapies [18].

We present clinical characteristics, neuroimaging findings, and outcomes data for 8 of the 10 patients with EEE diagnosed during the 2019 outbreak in Michigan, identified in 6 counties clustered in southwest Michigan. These data highlight the devastating burden of disease for affected patients and identify a critical need for improved diagnostic methods to facilitate clinical care and the development of EEE therapies.

METHODS

Cases were identified by treating physicians at 4 hospitals in southwest Michigan in Berrien, Calhoun, and Kalamazoo counties and occurred in patients residing in these counties, in addition to Van Buren and Cass counties. After approval of a protocol for informed consent by participating clinical sites and the local institutional review board (IRB), 8 patients consented to inclusion in the study. Patients included met 2015 CDC criteria for confirmed neuroinvasive arboviral disease [19], including a clinical syndrome of central or peripheral neurologic dysfunction and the absence of a more likely clinical explanation, as well as EEEV-specific molecular or serologic evidence of infection on CSF and/or serum testing, consisting of any of the following: (1) isolation of virus from, or demonstration of specific EEEV antigen or nucleic acid in clinical specimens, (2) ≥4-fold change in EEEV-specific quantitative antibody titers in paired serum samples, (3) EEEV-specific immunoglobulin (Ig) M in serum with confirmatory EEEV-specific neutralizing antibodies in the same or a later specimen, or (4) EEEV-specific IgM antibodies in CSF or serum samples.

Clinical, demographic, outcomes, and radiographic data were abstracted using record review and deposited into a secure database (REDCap [Research Electronic Data Capture]). Anonymized radiographic images were reviewed by a neuroradiologist blinded to previous diagnostic interpretations for localization and character of imaging abnormalities. Formalin-fixed brain autopsy specimens were sectioned, stained, and examined according to routine clinical protocols. Commercial laboratory testing for EEE was performed at Mayo Clinic Laboratories for 5 patients and at Associated Regional and University Pathologists Laboratories for 1. All patients with a diagnosis based on serologic results underwent a confirmatory plaque reduction neutralization test (PRNT) at the Michigan Department of Health and Human Services laboratory. In 1 patient, reverse-transcription polymerase chain reaction (RT-PCR) for EEEV RNA was performed at the CDC Division of Vector-Borne Diseases (Fort Collins, Colorado).

Written consent was obtained from the patient or legally authorized representative. Local IRB review of this retrospective case series determined its exempt status (IRB no. WMed-2019-0537).

RESULTS

Demographics

Data were collected from 8 patients, none of whom have been reported elsewhere (Table 1). All patients initially presented to care during August 2019 and were male adults, with a median age of 64 years (range, 54–78 years). A history of mosquito

Table 1. Clinical Description and Outcomes in Patients With a Diagnosis of Eastern Equine Encephalitis

Patient	Age, y	Sex	Chief Symptom	Other Symptoms	Medical History	Examination Findings	Exposures	Outcome
1	68	Male	Confusion	Chest pain, fevers/chills, nausea, diaphoresis	Hypertension, systolic heart failure, hyperlipidemia, nephrolithiasis	Nuchal rigidity, inattention, disorientation	Lakeside residence	Death (9 d after discharge)
2	72	Male	Generalized weakness	Tremors, hip pain, confusion	Dementia, BPH	Right-sided facial droop, myoclonic jerks, asymmetrical hand tremor	Lakeside residence	Death (hospital d 5)
3	63	Male	Obtunded	NA	Hypertension, NIDDM, hyperlipidemia, stroke, seizure disorder	Unintelligible vocalizations	Working in barn	Death (hospital d 12)
4	63	Male	Seizure	Fever Headache	Hypertension, atrial fibrillation, prostate cancer—no active disease	Disorientation, inattention Amnesia	Camping; resided near wetland	Survival; mild sequelae
5	64	Male	Seizure	Confusion, fever, speech difficulty	BPH, vitamin D deficiency	Aphasia, amnesia	Camping; bird sanctuary	Death (hospital d 10)
6	78	Male	Dyspnea	Nausea, dizziness, fatigue, fever	Hypertension, NIDDM, coronary artery disease, systolic heart failure, hyperlipidemia, melanoma—no active disease	Tachypnea	Golfing, yard work	Death (27 d after discharge)
7	57	Male	Confusion	Fever, vomiting, lethargy	Obesity, ankylosing spondylitis—no recent treatment	Nuchal rigidity, hyperreflexia, inattention, dysarthria	Agricultural worker	Survival, severe sequelae
8	54	Male	Fever	Polydipsia, polyuria, myalgias	Hypertension, IDDM, peripheral arterial disease	Rigors, tachycardia	Agricultural worker	Death (61 d after discharge)

Abbreviations: BPH, benign prostatic hyperplasia; IDDM, insulin-dependent diabetes mellitus; NA, not applicable; NIDDM, non–insulin-dependent diabetes mellitus.

exposure or physical examination evidence of arthropod bites was elicited in 7 patients, and all patients were found to have had significant outdoor exposures (Table 1). Six resided outside municipal jurisdictions, 4 either resided near inland bodies of water or had visited them within the 2 weeks before admission, and 1 had visited a bird sanctuary.

Clinical Characteristics

Symptoms began 1–7 days (median, 1 day) before clinical presentation, while 1 patient was found obtunded and thus had an unknown timing of symptom onset. Confusion or altered level of consciousness was the chief symptom in 3 patients, while 2 patients presented with a chief symptom of seizure. Six patients reported fever at presentation, and none of the patients had premorbid neurologic deficits. None had a diagnosed primary immunocompromising condition, and none were receiving immunomodulatory or antineoplastic therapy; 3 patients had preexisting diabetes mellitus. Initial physical examination found an altered level of consciousness in 5 patients, and 3 patients had a seizure on the day of presentation. Three patients were febrile (≥38°C) at presentation, and all 8 were febrile within 24 hours. Tachycardia (pulse rate >100/min) was present in 6, and tachypnea (respiration rate ≥20/min) in 5. Six patients had abnormal neurologic examination findings, ranging from focal findings, including facial droop and myoclonic jerks, to aphasia and amnesia (5 patients). The most common hospital admitting diagnoses were stroke and acute encephalopathy (in 2 patients each); the other admitting diagnoses were meningoencephalitis, aseptic meningitis, undifferentiated sepsis, and community-acquired pneumonia.

Laboratory Findings

Initial laboratory investigation found peripheral leukocytosis (white blood cell count $>11 \times 10^9$/L) in 6 patients, all with a neutrophilic predominance. Hyponatremia was not seen at presentation. All patients underwent lumbar puncture within 24 hours of presentation, and initial CSF findings (Table 2) universally showed elevated protein levels and a nucleated pleocytosis; the differential was predominantly polymorphonuclear in the majority of patients (6 of 8), consistent with previously reported data [16]. Hypoglycorrhachia was not seen. In all 4 patients with initially polymorphonuclear-predominant pleocytosis who underwent follow-up lumbar puncture, the follow-up CSF sample demonstrated a transition to lymphocytic predominance. One patient (patient 8) was also seropositive for California group LaCrosse virus (LACV) based on CSF and peripheral blood IgG findings, with

Table 2. Cerebrospinal Fluid Characteristics

Patient	Time Since Symptom Onset, d	Initial LP Results							Subsequent LP Results						
		RBCs/μL	WBCs/μL	% PMN	% Mononuclear[a]	Protein, mg/dL	Glucose, mg/dL	Time Since Initial LP, d	RBCs/μL	WBCs/μL	% PMN	% Mononuclear[a]	Protein, mg/dL	Glucose, mg/dL
1	1	<2000	656	75	25	117	87	5	<2000	81	0	100	166	59
2	2	<2000	1644	83	17	140	57	…	…	…	…	…	…	…
3	…[b]	<2000	129	12	88	182	70	7	<2000	60	0	100	233	105
4	2	<2000	99	56	44	201	107	5	<2000	60	0	100	87	80
5	4	2950	340	83	17	141	70	…	…	…	…	…	…	…
6	3	<2000	254	54	46	151	105	…	…	…	…	…	…	…
7	1	0	404	45	55	142	65	12	5	35	0	100	86	75
8	7	195	2822	57	38	193	177	11	0	138	0	100	116	93
Median	…	…	372	56.5	41	146.5	78.5	…	…	…	…	…	…	…

Abbreviations: LP, lumbar puncture; PMN, polymorphonuclear; RBCs, red blood cells; WBCs, white blood cells.

[a]Including lymphocytes and monocytes.

[b]Unknown.

corresponding PRNT titers of 1:256 in CSF and 1:20 480 in blood; the follow-up CSF PRNT titer 2 weeks later was 1:512, while the peripheral blood titer remained unchanged.

Imaging Findings

All patients underwent computed tomography (CT) at presentation, and 7 patients (87.5%) underwent subsequent magnetic resonance (MR) imaging, at a median of 2 days (range, 0–15) after presentation (Figure 1). The initial head CT study was without evidence of hemorrhage, ischemic infarct, or mass effect in all patients. However, abnormal findings were ultimately seen on ≥1 imaging study in all patients, though the findings were much more conspicuous on MR images. At CT, hypoattenuation of the affected structures was the only finding. With MR imaging, T2-weighted fluid-attenuated inversion recovery hyperintensity of the affected structures was the primary finding, frequently but not always with corresponding diffusion restriction. No parenchymal enhancement was present on any of the postcontrast MR imaging sequences. None of the patients had findings of parenchymal hemorrhage, and 1 had an isolated abnormality of the brainstem. All other patients had abnormalities of the cerebrum, involving predominantly the basal ganglia and thalami and to a lesser extent the cerebral cortex and periventricular white matter. Two patients had mild leptomeningeal enhancement associated with areas of cortical involvement, though it should be noted that not all patients undergoing MR imaging had postcontrast imaging. In general, neuroimaging findings were dynamic and progressive over time, with serial studies showing progressive involvement of further regions of the brain.

EEE Diagnostics

The clinical diagnosis of EEE was based on the presence of CSF EEEV IgM antibodies in 6 of 8 cases (Table 3). In 1 case, EEEV infection could be detected only with CSF RT-PCR, and in the other, EEE was not initially clinically suspected and was identified only after peripheral blood serologic results were positive for EEEV neutralizing antibodies. Notably, commercial reference laboratory EEEV serology was performed on the initial CSF specimen in 5 patients, and results were negative for EEE IgG and IgM in all 5. Public health laboratory testing for EEEV IgM was performed on the same initial CSF specimen in 3 cases, with positive results in 2 cases. Two patients with negative results of initial commercial CSF serology underwent follow-up lumbar puncture testing 5 and 9 days later, at which time both were found to be positive for EEEV IgM and IgG at the same laboratory. In 1 patient EEE could not be diagnosed with commercially available CSF serologic testing despite lumbar punctures performed on days 2 and 7 of symptoms, but it was diagnosed via public health laboratory identification of EEEV IgM with the initial, day 2 CSF specimen. Finally, a single patient had negative commercial and

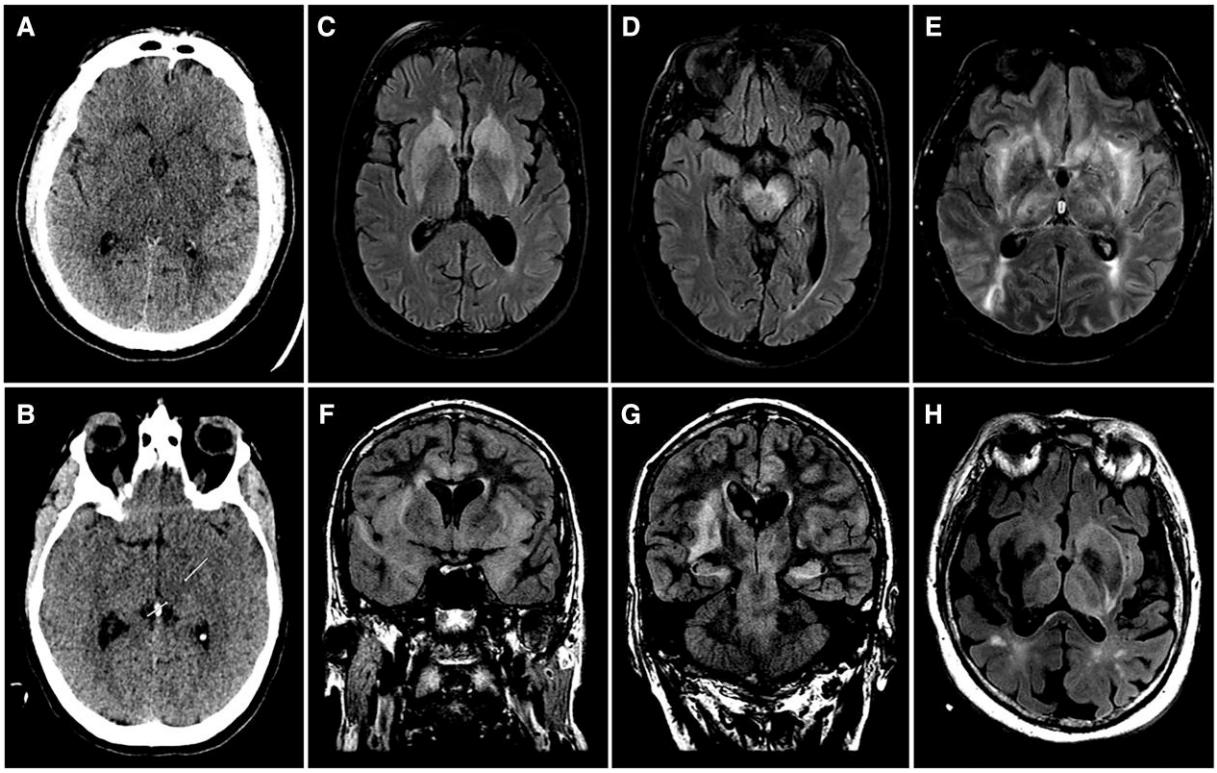

Figure 1. Eastern equine encephalitis neuroimaging. Representative cross-sections are shown from computed tomography (*A, B*) and magnetic resonance (MR) imaging (*C–H*). *A*, Diffuse symmetric hypoattenuation of the bilateral basal ganglia, thalami, and adjacent deep white matter. *B*, Subtle hypoattenuation in the left thalamus (*arrows*). *C, D*, MR images from same patient showing symmetric fluid-attenuated inversion recovery (FLAIR) hyperintensity in the basal ganglia, thalami, and brainstem. *E*, FLAIR hyperintensity in basal ganglia, thalami, adjacent deep white matter, and periventricular white matter, as well as asymmetric areas of bilateral cortical hyperintensity. *F, G*, Coronal sections from same patient with symmetric hyperintensity of basal ganglia, thalami, and brainstem, as well as medial temporal lobes and cingulate gyri. Asymmetric hyperintensity is seen in the cerebral cortex and periventricular white matter. *H*, Asymmetric hyperintensity of basal ganglia, thalami, and adjacent deep white matter, with chronic right basal ganglia infarct.

public health serologic CSF results, and the diagnosis was made only with RT-PCR testing of the same CSF specimen at the CDC Division of Vector-Borne Diseases. Altogether, diagnosis ultimately relied on public health laboratory testing in 6 of 8 cases, while 2 cases were diagnosed based on testing at commercial reference laboratories.

The time to diagnosis in all 8 patients was prolonged, with the diagnosis was not made until late in the clinical course. The median time between patient presentation and receipt of information that allowed diagnosis by clinicians was 24.5 days (range, 13–38 days). Only 2 patients received a diagnosis during their index hospitalization, and 3 had died by the time diagnostic information was received.

Outcomes

Three patients (37.5%) died during the index hospitalization. By 18-month follow-up, 3 additional patients had died after being discharged with severe neurologic deficits, and their deaths were assessed as EEE attributable. One patient survived with severe neurologic deficits requiring 24-hour care, and 1 patient has recovered to premorbid levels of functioning. The overall EEE-attributable mortality rate for cases reported in this convenience sample is 6 of 8 patients, or 75%.

Postmortem Examination

A limited postmortem brain examination was performed on a single patient (patient 5). Multifocal leptomeningeal congestion was noted at gross examination, along with an ill-defined region of congested vasculature within the anterior right frontal subcortical white matter measuring 1.5 cm, without gross intraparenchymal hemorrhage. On microscopic examination, widespread neuronophagia was seen, with microglial clusters and abundant clusters of macrophages scattered throughout the parenchyma (Figure 2), as reported elsewhere [20]. Perivascular mononuclear cell cuffing was seen throughout the brain parenchyma and focally within the leptomeninges, as well as scattered regions of vessel wall infiltration by mixed inflammatory cells, early vessel wall necrosis, and perivascular extravasation of erythrocytes. Widespread eosinophilic neurons were present throughout the brain parenchyma, with frequent macrophages throughout Ammon's horns bilaterally. Sections of the basal ganglia, hippocampi, and thalamus showed overall normal architecture.

Table 3. Diagnostic Testing for Eastern Equine Encephalitis Virus[a]

	CSF Results for 1st LP					CSF Results for 2nd LP				CSF Results for 3rd LP				Peripheral Blood Results			
Patient	Time Since Symptom Onset, d	IgM	IgG	PRNT	RT-PCR	Time Since Symptom Onset, d	IgM	IgG	PRNT	Time Since Symptom Onset, d	IgM	IgG	PRNT	Time Since Symptom Onset, d	IgM	IgG	PRNT	Time to Diagnosis, d[b]
1	1	*<1:10*	*<1:10*	…	…	10	**Pos**[c]	…	…	18	…	…	**1:16**[c]	15	*<1:10*	*>1:40*[c]	…	23
					…			…	…		*>1:10*[c]	*>1:40*[c]	…					
2	2	**Pos**[c]	…	**1:2**[c]	…	…	…	…	…	…	…	…	…	…	…	…	…	29
		<1:10	*<1:10*	…	…	…	…	…	…	…	…	…	…	…	…	…	…	
3	1[d]	*<1:10*	*<1:10*		…	8[d]	**Pos**[c]	…	**1:64**[c]	…	…	…	…	7[d]	*<1:10*	*>1:40*[c]	…	13
					…		*>1:10*[c]	*>1:40*[c]	…	…	…	…	…					
4	2	**Pos**[c]	…	**ND**	…	7	*<1:10*	*<1:10*		…	…	…	…	19	…	…	**1:320**[c]	22
		<1:10	*<1:10*	…	…					…	…	…	…		…	…		
5	4	**ND**	…	…	**Pos**[c]	…	…	…	…	…	…	…	…	…	…	…	…	32
		<1:1	*<1:1*	…		…	…	…	…	…	…	…	…	…	…	…	…	
6	…	…	…	…	…	…	…	…	…	…	…	…	…	18	…	…	**1:10240**[c]	20
	…	…	…	…	…	…	…	…	…	…	…	…	…		*>1:10*[c]	*>1:40*[c]	…	
7	1	**Pos**[c]	…	**1:4**[c]	…	…	…	…	…	…	…	…	…	…	…	…	…	26
8	7	…	…	**<1.2**	…	26	…	…	**1:512**[c]	…	…	…	…	13	…	…	**1:640**[c]	38
		<1:1	*<1:1*	…	…		*1:1*[c]	*1:102*[c]	…	…	…	…	…		…	…		
Median	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	24.5

Abbreviations: CSF, cerebrospinal fluid; Ig, immunoglobulin; LP, lumbar puncture; ND, not detected; Pos, positive; PRNT, plaque reduction neutralization test; RT-PCR, reverse-transcription polymerase chain reaction.

[a]All diagnostic tests for eastern equine encephalitis virus are listed for each patient. Results in italics derived from commercial laboratory testing; results in bold derived from public health laboratory testing.

[b]Time elapsed between clinical presentation and receipt of information conferring the diagnosis by treating clinicians.

[c]Abnormal results.

[d]Days since hospital presentation.

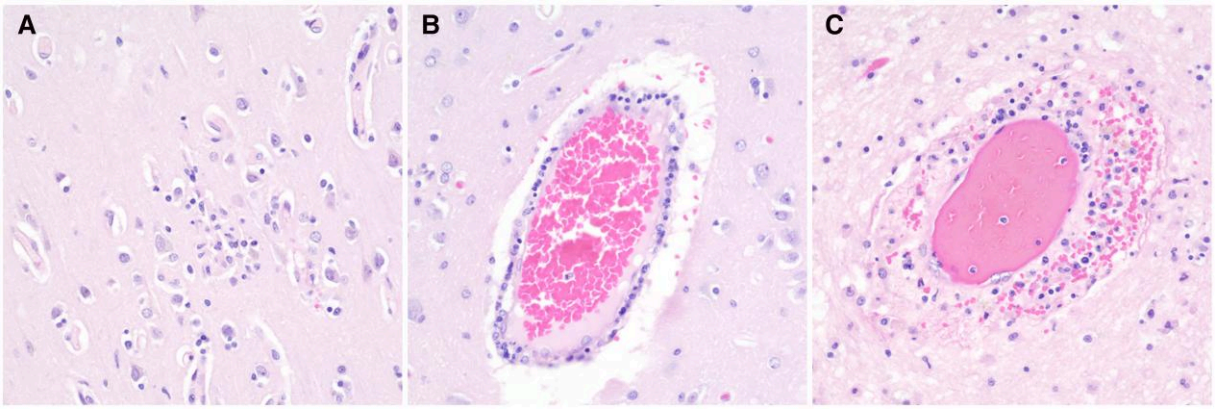

Figure 2. Neuropathologic findings in eastern equine encephalitis. Representative photomicrographs of sections stained with hematoxylin-eosin are shown at ×200 magnification. *A*, Multifocal hypercellularity with neuronophagia and microglial nodules. *B*, Scattered intraparenchymal vessels exhibiting mononuclear cell cuffing. *C*, Occasional intraparenchymal vessels infiltrated by mixed inflammatory cells, with early vessel wall necrosis.

DISCUSSION

The care of patients with EEE continues to pose significant diagnostic and management challenges. The series presented here recapitulates previous findings in the literature regarding EEE clinical presentation and outcomes, including a high mortality rate and morbidity rate among survivors, imaging abnormalities within the basal ganglia and thalami, and a predominantly neutrophilic pleocytosis at initial CSF analysis. In contrast to previous reports, hyponatremia was not seen at presentation [14]. We also illustrate the diagnostic difficulties facing clinicians caring for patients with EEE. Despite prompt workup including lumbar puncture and neuroimaging, the time to diagnosis for patients presenting with EEE was prolonged, typically requiring >3 weeks from the time of hospital presentation. This long window of uncertainty for patients facing a life-threatening illness complicates clinical care and also poses a formidable barrier to the development of therapeutics, standing athwart considerable recent progress in their preclinical development [21].

Only a minority of the patients in the current series received a hospital admitting diagnosis consistent with EEE, and the in-depth description of diagnostic studies presented here identifies poor sensitivity of serologic testing on the initial CSF sample as a barrier to a timely diagnosis. The disparate hospital admitting diagnoses highlight the need for enhanced clinical suspicion in endemic regions during arboviral transmission season. Patients in this series nonetheless underwent prompt lumbar puncture and, with a single exception, prompt CSF arboviral serologic testing.

The prolonged time to receipt of diagnostic information by treating clinicians is in part attributable to poor sensitivity of the initial CSF serology, particularly with commercial laboratory testing. Notably, public health laboratory testing of CSF specimens using microsphere immunoassay appeared to show greater sensitivity with the initial CSF specimen than commercially available assays, which use a cell-based, indirect fluorescent antibody technique. This concern was also identified in a previous report [15]. Thus, more timely diagnosis of EEE may be achieved by wider use of improved serologic techniques. The microsphere immunoassay used here was developed by the CDC and uses arboviral antigen-decorated beads with fluorescent detection of antibody via the Bio-Plex platform [22, 23].

We note that public health CSF serologic testing results were also negative with the initial CSF specimen in 2 cases, 1 of which was ultimately diagnosed with RT-PCR testing of the same CSF specimen. Molecular viral RNA detection was not commercially available to treating clinicians and was obtained at a national public health laboratory (CDC Division of Vector-Borne Diseases) in only 1 patient, after CSF serologic results returned negative. The sensitivity of serologic diagnosis may be inherently limited early during infection, particularly in EEE, where clinical progression is frequently rapid.

Further study of the utility of RT-PCR testing by commercial and/or state-level public health laboratories is warranted to determine whether its use would improve the time to diagnosis for EEE patients. Diabetes may affect the humoral immune response [24], and 2 patients with diabetes had negative results with initial CSF serology. Further research on the impact of diabetes on the sensitivity of serologic testing in arboviral infections is also needed. Finally, peripheral blood arboviral serology was underused during the workup of the patients presented here, particularly at the time of presentation. Prompt use of peripheral blood serology may shorten the time to clinical diagnosis.

While basal ganglia and thalamus abnormalities were ultimately identified on neuroimaging in most patients, these abnormalities were not typically present at the time of

presentation, making them unreliable as an initial diagnostic clue. Notably, basal ganglia abnormalities were not identified in 2 patients; 1 had only isolated brainstem involvement, and 1 had an isolated abnormality in the thalamus, albeit in the absence of accompanying MR imaging studies. The initial head CT was insensitive to diagnostic clues, with serial scans being necessary to disclose abnormalities. Thus, while the characteristic pattern of basal ganglia involvement continues to be a useful diagnostic marker, a subset of patients present without this finding, necessitating high clinical suspicion in endemic regions at times when arboviral transmission is likely.

The patients in this series were overwhelmingly male and were significantly older than the Michigan population median of 40.2 years [25], consistent with previously reported data at the national [7] and state [26] levels. This suggests that immunosenescence may predispose to adverse outcomes of EEEV infection. While differential exposure to mosquito vectors likely plays a role in explaining the male preponderance for severe EEEV infections, experience with other viral infections, including severe acute respiratory syndrome coronavirus 2, lends credence to the possibility that sex differences may influence susceptibility to poor EEEV outcomes [27]. The incidence of EEE has classically shown a bimodal distribution vis-à-vis age [4], in contrast to the unimodal, older patient group identified here. While this may be a consequence of incomplete study recruitment and/or missed clinical diagnoses in pediatric patients (state-level data did identify a single pediatric case in 2019 [26]), national data also identified a predominance of aged patients [7]. Further research is needed to understand the wide interindividual variability in outcomes after EEEV infection, ranging from asymptomatic to rapidly fatal.

IVIG has been used anecdotally for EEEV [16, 28, 29], and a retrospective analysis of patients with EEE in New England between 2005 and 2019, more than half of whom received IVIG, identified a correlation between time to IVIG administration and severity of long-term disability [16]. IVIG was not used in the care of patients presented here, and its efficacy in treating EEE is unclear. EEEV-neutralizing antibodies may be present in some IVIG preparations, depending on seroprevalence among donors, which is poorly defined and is expected to vary based on geography. IVIG also has immunomodulatory properties, including down-regulation of macrophage and T-cell function [30], which may be relevant to EEE pathogenesis. The heterogeneity in EEE treatment underscores the need for further study of the role of IVIG and other immunomodulatory agents in this condition.

One patient in the current series was seropositive for LACV, which is rarely identified in humans; neuroinvasive LACV was diagnosed only twice in Michigan between 2011 and 2020 [31]. This patient reported a history of fatigue, myalgia, and fever beginning 7 days before his presentation with acute encephalopathy and seizures, the longest such symptomatic interval before presentation in the series. Initial CSF serology did not identify LACV IgM but did find plaque-neutralizing IgG. Peripheral blood PRNT titers were already high on illness day 13 and had not changed at reassessment on days 22 and 35. Because serologic cross-reactivity between LACV, a bunyavirus, and EEEV, a togavirus, is unlikely, these diagnostic data are consistent with previous infection by LACV. Exposure to 2 rarely diagnosed arboviral infections in the same patient is unexpected but has been reported elsewhere [32], and in this case was presumably due to extensive agricultural occupational exposure to mosquito vectors in an area with high levels of epizootic spillover. Because nonneuroinvasive arboviral infections are rarely identified, their incidence is poorly understood and should be investigated with further population-level studies, such as serosurveys.

EEE remains a formidable public health and clinical challenge, and its impact seems likely to increase in coming years. Because the initial clinical presentation of EEE can be nonspecific, attention to improving diagnostic modalities may allow for prospective testing of immunomodulatory and antiviral treatment strategies, and may ultimately improve clinical care for affected patients.

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