Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis: Consensus Statement of the International Encephalitis Consortium


1Johns Hopkins Encephalitis Center, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland; 2Warren Alpert Medical School of Brown University, Providence, Rhode Island; Departments of 3Medicine (Infectious Diseases) and 4Preventive Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; 5Division of Infectious Diseases, Department of Medicine, University of Michigan Medical School, Ann Arbor; 6Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 7Division of Infectious Diseases, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Ontario, Canada; 8Infectious Diseases Department, CHU and University 1, Grenoble, France; 9Division Infectious Diseases, French Institute for Public Health Surveillance, Saint-Maurice, France; 10National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba; 11Global Alliance For Rabies Control, Manhattan, Kansas; 12Waterborne Disease Prevention Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Departments of 13Neurology and 14Medicine, Boston University School of Medicine, Massachusetts; Departments of 15Pediatrics, 16Microbiology, 17Medicine, and 18Neurosurgery, University of Alabama at Birmingham; 19Centro Clinical School, University of Sydney, New South Wales, Australia; 20Virus Reference Department, Public Health England, London; 21Sydney Emerging Infectious Diseases and Biosecurity Institute, and 22The Children’s Hospital Westmead Clinical School, University of Sydney, New South Wales, and 23Hunter New England Population Health, Wallsend, New South Wales, Australia; 24Division of Infectious and Immunology, University College London, England; 25University of Newcastle, and 26Hunter Medical Research Institute, Wallsend, New South Wales, Australia; 27Department of Medicine (Neurology) and Medical Microbiology, University of Manitoba, Winnipeg, Canada; 28Department of Viral Encephalitis and Arbovirus, State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, People’s Republic of China; and 29Division of Communicable Disease Control, California Department of Public Health, Richmond

Background. Encephalitis continues to result in substantial morbidity and mortality worldwide. Advances in diagnosis and management have been limited, in part, by a lack of consensus on case definitions, standardized diagnostic approaches, and priorities for research.

Methods. In March 2012, the International Encephalitis Consortium, a committee begun in 2010 with members worldwide, held a meeting in Atlanta to discuss recent advances in encephalitis and to set priorities for future study.

Results. We present a consensus document that proposes a standardized case definition and diagnostic guidelines for evaluation of adults and children with suspected encephalitis. In addition, areas of research priority, including host genetics and selected emerging infections, are discussed.

Conclusions. We anticipate that this document, representing a synthesis of our discussions and supported by literature, will serve as a practical aid to clinicians evaluating patients with suspected encephalitis and will identify key areas and approaches to advance our knowledge of encephalitis.

Keywords. encephalitis; guidelines; viral; autoimmune; host genetics.

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Correspondence: Arun Venkatesan, MD, PhD, Johns Hopkins University School of Medicine, 600 N Wolfe St, Meyer 6-113, Baltimore, MD 21287 (avenkat2@jhmi.edu).
Advances in encephalitis are hampered by the rarity and heterogeneity of cases, highlighting the need for a collaborative international approach. In March 2012, the International Encephalitis Consortium held a meeting in Atlanta to discuss recent advances in encephalitis and to set priorities for future study. This consortium is an ad-hoc committee begun in 2010 with members from the Americas, Europe, Australia, Africa, and Asia. The mission of the consortium is to advance knowledge of the causes, diagnostic strategies, treatment, and outcome of encephalitis, and to implement interventions based upon this knowledge. Topics discussed at the meeting included: (1) standardization of a case definition for encephalitis, (2) development of practical diagnostic algorithms for evaluation of patients, (3) the role of host genetics in encephalitis, and (4) priorities for the study of selected emerging infectious diseases. Here we present a consensus document that synthesizes our discussions and recent literature, with the goals of aiding clinicians evaluating patients with suspected encephalitis and of identifying priorities and approaches to advance knowledge of encephalitis.

**PRIORITY 1: CASE DEFINITION**

Encephalitis is defined as inflammation of the brain parenchyma associated with neurologic dysfunction [1]. Although pathologic examination and testing of brain tissue is considered to be the “gold standard” diagnostic test for this syndrome, this is rarely done premortem due to potential morbidity associated with an invasive neurosurgical procedure. In the absence of pathologic confirmation, encephalitis has previously been defined on the basis of selected clinical, laboratory, electroencephalographic, and neuroimaging features [2–7] (Supplementary Table 1). One of the most widely used case definitions for encephalitis, developed by the Brighton Collaboration Encephalitis Working Group [6], standardizes reporting of post-immunization neurologic events. However, whether this definition is applicable to the diagnosis of infectious or autoimmune encephalitis, as well as the relative sensitivity and specificity of the varying levels of diagnostic accuracy of this definition, is unknown.

Further complicating development of a cohesive case definition for encephalitis is the clinical overlap between encephalitis and encephalopathy, terms often used interchangeably in the literature but that may represent distinctive pathophysiologic processes. Encephalopathy refers to a clinical state of altered mental status, manifesting as confusion, disorientation, behavioral changes, or other cognitive impairments, with or without inflammation of brain tissue. Encephalopathy without inflammation can be triggered by a number of metabolic or toxic conditions but may also be associated with specific infectious agents, such as *Bartonella henselae* [8–10] or influenza virus [11–14].

In contrast, encephalitis is characterized by brain inflammation as a consequence of direct infection of the brain parenchyma, a post-infectious process such as acute disseminated encephalomyelitis (ADEM) [6, 15], or a noninfectious condition such as anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis [16, 17]. In the absence of pathologic evidence of brain inflammation, an inflammatory response in the cerebrospinal fluid (CSF) or the presence of parenchymal abnormalities on neuroimaging are often used as surrogate markers of brain inflammation. However, encephalitis can occur without significant CSF pleocytosis or demonstrable neuroimaging abnormalities [18–21].

Development of a standardized case definition for encephalitis and encephalopathy of presumed infectious etiology is important for epidemiological surveillance, clinical research, and outbreak investigations. Implementation of a case definition broadly applicable to regions with substantially different resources and surveillance capacities facilitates investigation of newly recognized or emerging causes of encephalitis. Because of the significant clinical overlap between encephalitis (infectious and noninfectious) and encephalopathy of presumed infectious etiology, the case definition is formulated to capture both syndromes.

Several caveats must be recognized regarding the proposed case definition. First, alteration in mental status is a required component (Major criterion; Table 1). It is recognized that some infections or conditions related to infections may cause central nervous system (CNS) dysfunction without affecting consciousness (eg, post-varicella cerebellar ataxia [22]), and our case definition would not capture these entities. Second, there is no restriction on the maximum duration of altered mental status, and therefore both acute causes of encephalitis as well as more subacute or chronic infectious conditions such as those caused by fungi or mycobacteria would meet the case definition. Third, several additional criteria are required to substantiate a diagnosis of encephalitis (Minor criteria; Table 1). Finally, the syndromic definition is viewed to be complementary to the diagnostic testing algorithm (see Priority 2: Diagnostic Algorithm section and Tables 2 and 3). Thus, while identification of an infection with an organism that is strongly associated with encephalitis from an appropriate biologic sample would confirm a clinical diagnosis of encephalitis, failure to identify a pathogen, as has been reported in >50% of cases of presumed encephalitis in some series [1, 5], would not exclude the diagnosis.

**Summary**

The proposed definition of encephalitis and encephalopathy of presumed infectious etiology was developed based on consensus expert opinion and review of available literature. We anticipate that validation using existing cohorts as well as additional prospective studies will be crucial in refining and improving the case definition for encephalitis.
**Table 1. Diagnostic Criteria for Encephalitis and Encephalopathy of Presumed Infectious or Autoimmune Etiology**

<table>
<thead>
<tr>
<th>Major Criterion (required):</th>
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<tr>
<td>Patients presenting to medical attention with altered mental status (defined as decreased or altered level of consciousness, lethargy or personality change) lasting ≥24 h with no alternative cause identified.</td>
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<table>
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<tr>
<th>Minor Criteria (2 required for possible encephalitis; ≥3 required for probable or confirmed a encephalitis):</th>
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<tr>
<td>Documented fever ≥38°C (100.4°F) within the 72 h before or after presentation b</td>
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<td>Generalized or partial seizures not fully attributable to a preexisting seizure disorder c</td>
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<td>New onset of focal neurologic findings</td>
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<td>CSF WBC count ≥5/cubic mm d</td>
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<td>Abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is either new from prior studies or appears acute in onset e</td>
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<tr>
<td>Abnormality on electroencephalography that is consistent with encephalitis and not attributable to another cause. f</td>
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Abbreviations: CNS, central nervous system; CSF, cerebral spinal fluid; EEG, electroencephalogram; RBC, red blood cell; WBC, white blood cell.

a Confirmed encephalitis requires one of the following: (1) Pathologic confirmation of brain inflammation consistent with encephalitis; (2) Defined pathologic, microbiologic, or serologic evidence of acute infection with a microorganism strongly associated with encephalitis from an appropriate clinical specimen (for examples, see references 1, 2); or (3) Laboratory evidence of an autoimmune condition strongly associated with encephalitis.

b Fever is a common finding in patients with acute encephalitis but is nonspecific. The requirement for objective documentation of fever within a restricted time frame of ≤72 h after hospitalization was chosen to exclude secondary health-care associated infections. It is recognized that fevers can occur as a result of a number of infections outside of the central nervous system that can cause encephalopathy, as well as with noninfectious entities that mimic encephalitis. It is also recognized that fever may fluctuate and, as such, objective fever may be lacking in patients with infectious encephalitis at the time of clinical assessment. Furthermore, immunosuppressed patients with encephalitis may not mount a fever.

c Seizures associated with encephalitis may be generalized, suggestive of global CNS dysfunction, or focal, indicating a localized process. Subclinical seizures may also occur and can be a cause of altered sensorium. Seizures associated with high temperatures are relatively common in young children and, if occurring in isolation, do not mandate evaluation for encephalitis. The major requirement for at least 24 h of altered mentation was selected to exclude the post-ictal state seen in patients with febrile seizures.

d CSF pleocytosis is suggestive of an inflammatory process of the brain parenchyma, meninges, or both (meningoencephalitis). The absence of CSF pleocytosis, however, does not exclude encephalitis. In particular, it is recognized that the CSF may be devoid of cells in immunocompromised patients (Fodor et al., Neurology 1998 51:554–59) or early in the course of infection (Weil et al, Clin Infect Dis 2002 34:1154–57; Mook-Kanamori et al., J Am Geriatr Soc 57:1514–15; Jakob et al., Crit Care Med 2012 40:1304–8). Conversely, the CSF profile with inflammation limited to the meninges may be indistinguishable from that in patients with encephalitis. In the majority of cases of encephalitis, however, the absolute number of leukocytes is <1000/mm^3 and lymphocytes typically predominate. To ensure adequate sensitivity of the definition, the group defined CSF pleocytosis as ≥5 WBC/mm^3. In cases where there are large numbers of red blood cells in the CSF, such as with a traumatic lumbar puncture, the following formula may allow correction of the WBC count: True CSF WBC = actual CSF WBC / (WBC in blood X 11:423).

Fever, CSF pleocytosis, and seizures associated with encephalitis are not mutually exclusive. Seizures may also occur in patients with CSF pleocytosis, and fever may be present in patients with CSF pleocytosis. CSF pleocytosis, seizures, and fever may all be present in the same patient.

e Seizures associated with encephalitis may be generalized, suggestive of global CNS dysfunction, or focal, indicating a localized process. Subclinical seizures may also occur and can be a cause of altered sensorium. Seizures associated with high temperatures are relatively common in young children and, if occurring in isolation, do not mandate evaluation for encephalitis. The major requirement for at least 24 h of altered mentation was selected to exclude the post-ictal state seen in patients with febrile seizures.

f EEG abnormalities reported in cases of encephalitis range from nonspecific generalized slowing to distinctive patterns suggestive of specific entities, including repetitive sharp wave complexes over the temporal lobes or periodic lateralizing epileptiform discharges in HSV-1 (Lai and Gragasoin J Clin Neuropsychol 1988 5:87–103) and bilateral synchronous periodic sharp and slow waves associated with subacute sclerosing panencephalitis (Gutierrez et al. Dev Med Child Neurol 2010 52:901–7). EEG abnormalities are frequently nonspecific and may be attributable to medications or metabolic abnormalities. The EEG may identify epileptiform discharges in the absence of clinical evidence of seizure activity (subclinical or nonconvulsive status epilepticus) as a cause of obtundation.

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**PRIORITY 2: DIAGNOSTIC ALGORITHM**

**Scope and Purpose**

Algorithms for the diagnosis of encephalitis may serve many purposes, including aiding clinicians in management of patients, standardizing evaluations for research, and facilitating public health disease surveillance. Several groups have recently provided reviews of diagnosis and management of encephalitis, with differing purposes and depth [1, 23–26]. Our primary goal was to develop a practical diagnostic algorithm for use by medical professionals worldwide in the initial evaluation of suspected encephalitis. In addition, we intended the algorithm to provide a standardized approach for use in collaborative, multi-center research studies. Etiologies that we focus on include those that (1) are more commonly identified, (2) may benefit from targeted therapies, or (3) are of particular public health significance. The algorithm is directed toward identification of specific infectious and autoimmune causes of encephalitis and therefore does not include a broad evaluation for mimickers of encephalitis or other causes of encephalopathy.
### ROUTINE STUDIES

**CSF**
- Collect at least 20 cc fluid, if possible; freeze at least 5–10 cc fluid, if possible
- Opening pressure, WBC count with differential, RBC count, protein, glucose
- Gram stain and bacterial culture
- HSV-1/2 PCR (if test available, consider HSV CSF IgG and IgM in addition)
- VZV PCR (sensitivity may be low; if test available, consider VZV CSF IgG and IgM in addition)
- Enterovirus PCR
- Cryptococcal antigen and/or India Ink staining
- Oligoclonal bands and IgG index
- VDRL

**SERUM**
- Routine blood cultures
- HIV serology (consider RNA)
- Treponemal testing (RPR, specific treponemal test)
- Hold acute serum and collect convalescent serum 10–14 d later for paired antibody testing

**IMAGING**
- Neuroimaging (MRI preferred to CT, if available)
- Chest imaging (Chest x-ray and/or CT)

**NEUROPHYSIOLOGY**
- EEG

**OTHER TISSUES/FLUIDS**
- When clinical features of extra-CNS involvement are present, we recommend additional testing (eg, biopsy of skin lesions; bronchoalveolar lavage and/or endobronchial biopsy in those with pneumonia/pulmonary lesions; throat swab PCR/culture in those with upper respiratory illness; stool culture in those with diarrhea); also see below

### CONDITIONAL STUDIES

**HOST FACTORS**
- Immunocompromised—CMV PCR, HHV6/7 PCR, HIV PCR (CSF); *Toxoplasma gondii* serology and/or PCR; MTB testing; fungal testing; WNV testing

**GEOGRAPHIC FACTORS**
- Africa—malaria (blood smear), trypanosomiasis (blood/CSF smear, serology from serum and CSF); dengue testing
- Asia—Japanese encephalitis virus testing; dengue testing; malaria (blood smear); Nipah virus testing (serology from serum and CSF; PCR, immunohistochemistry, and virus isolation in a BSL4 lab can also be used to substantiate diagnosis)
- Australia—Murray Valley encephalitis virus testing; Kunjin virus testing; Australian Bat Lyssavirus (ABL) testing
- Europe—Tick-borne encephalitis virus (serology); if Southern Europe, consider WNV testing; Toscana virus testing
- Central and South America—dengue testing; malaria (blood smear); WNV, Venezuelan equine encephalitis testing
- North America—Geographically appropriate arboviral testing (eg, WNV, Powassan, LaCrosse, Eastern Equine Encephalitis viruses; Lyme (serum ELISA and Western blot)

**SEASON AND EXPOSURE**
- Summer/Fall: Arbovirus and tick-borne disease testing
- Cat (particularly if with seizures, paucicellular CSF)—Bartonella antibody (serum), ophthalmologic evaluation
- Tick exposure—tick borne disease testing
- Animal bite/bat exposure—rabies testing
- Swimming or diving in warm freshwater or nasal/sinus irrigation—*Naegleria fowleri* (CSF wet mount and PCR)

**SPECIFIC SIGNS AND SYMPTOMS**
- Psychotic features or movement disorder—anti-NMDAR antibody (serum, CSF); rabies testing; screen for malignancy, Creutzfeld-Jakob disease
- Prominent limbic symptoms—Autoimmune limbic encephalitis testing; HHV6/7 PCR (CSF); screen for malignancy
- Rapid decompensation (particularly with animal bite history or prior travel to rabies-endemic areas)—rabies testing
- Respiratory symptoms—*Mycoplasma pneumoniae* serology and throat PCR (if either positive, then do CSF PCR); respiratory virus testing
- Acute flaccid paralysis—Arbovirus testing; rabies testing
- Parkinsonism—Arbovirus testing; *Toxoplasma* serology
- Nonhealing skin lesions—*Balamuthia mandrillaris*, *Acanthamoeba* testing
Laboratory Features

- Elevated transaminases—Rickettsia serology, tick-borne diseases testing
- CSF protein >100 mg/dL, or CSF glucose <2/3 peripheral glucose, or lymphocytic pleocytosis with subacute symptom onset—MTB testing, fungal testing
- CSF protein >100 mg/dL or CSF glucose <2/3 peripheral glucose and neutrophilic predominance with acute symptom onset and recent antibiotic use—CSF PCR for S. pneumoniae and N. meningitidis
- CSF eosinophilia—MTB testing, fungal testing, Baylisascaris procyonis antibody (serum); Angiostrongylus cantonensis and Gnathostoma sp. testing
- RBCs in CSF—Naegleria fowleri testing
- Hyponatremia—anti-VGKC antibody (serum); MTB testing

Neuroimaging Features

- Frontal lobe—Naegleria fowleri testing (CSF wet mount and PCR)
- Temporal lobe—VGKC antibodies (serum and CSF); HHV 6/7 PCR (CSF)
- Basal ganglia and/or thalamus—Arbovirus testing; MTB testing
- Brainstem—Arbovirus testing; Listeria PCR (if available); Brucella antibody (serum); MTB testing
- Cerbellum—EBV PCR (CSF) and serology
- Diffuse cerebral edema—Respiratory virus testing
- Space occupying and/or ring-enhancing lesions—MTB testing, fungal testing, Balanuthia mandrillaris and Acanthamoeba testing; Toxoplasma serology
- Hydrocephalus and/or basilar meningeal enhancement—MTB testing, fungal testing; respiratory virus testing

Infarction or hemorrhage—MTB testing; fungal testing; respiratory virus testing

Abbreviations: ABLV, Australian bat lyssavirus; BSL4, biosafety level 4; CNS, central nervous system; CSF, cerebral spinal fluid; CT, computed tomography; EBV, Epstein-Barr virus; EEG, electroencephalography; ELISA, enzyme-linked immunosorbent assay; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MRI, magnetic resonance imaging; MTB, Mycobacterium tuberculosis; PCR, polymerase chain reaction; VDRL, Venereal Disease Research Laboratory; VGKC, voltage-gated potassium channel; VZV, varicella-zoster virus; RBC, red blood cell; WBC, white blood cell; WNV, West Nile virus.

a This table is not intended to encompass all causes of encephalitis, nor all epidemiological or laboratory-based risk factors. We recommend using this table as a guideline for initial management of acute encephalitis in adults. For additional information, we recommend consulting Tunkel et al. 2008, Steiner et al. 2010, Solomon et al. 2012 (see references). Consultation with local health authorities is also recommended.

b MTB testing includes CSF smear for acid-fast bacilli and CSF mycobacterial culture along with one or more of the number of MTB PCR tests for CSF now commercially available. Sensitivity of smear and culture increases with the volume of CSF analyzed; we recommend consulting with the laboratory regarding optimal volumes of CSF to be analyzed. Given the varying sensitivity of these tests, systemic MTB testing including tuberculin skin test (may be negative) or interferon gamma release assay, stains and cultures from sputum, and tissue from biopsies from any potential systemic sites of infection.

c Fungal testing should be tailored to specific geographic region and prior travel history/place of residence, and typically consists of serology, antibody testing from urine and/or CSF, and cultures from blood and CSF.

d Arbovirus testing should be tailored to specific geographic region and typically consists of IgG and IgM from serum and CSF; PCR (serum, CSF) can be performed for select arboviruses (ie, WNV, California serogroup viruses), and is particularly useful in immunocompromised patients.

rabies/ABLV testing includes serologic analysis of serum and CSF; virus isolation or RT-PCR from saliva; tests for viral antigen or histopathology on either a brain biopsy or full-thickness biopsy of the nape of the neck. Testing should be conducted in concert with a local or regional public health department.

tick-borne disease testing should be tailored to specific geographic region and typically consists of serology (ie, Borrelia, Ehrlichia, Rickettsia sp., Anaplasma phagocytophilum, TBEV), and blood PCR (Ehrlichia, Anaplasma).

Naegleria fowleri, Balanuthia mandrillaris, and Acanthamoeba spp. testing is only available at specialized laboratories (eg, CDC) and includes serum immunofluorescence assay, immunohistochemistry on brain or other tissue and PCR testing on brain or other tissue and CSF. In addition, CSF wet mount is recommended for Naegleria fowleri testing. Brain tissue from affected region offers optimal sensitivity and specificity but other specimens can be tested.

Autoimmune limbic encephalitis evaluation includes testing for antibodies to VGKC (most commonly identified cause in adults), GAD, AMPA receptor, GABA<sub>a</sub> receptor, mGlur5, Hu, CV2, Ma2, and phosphine.

Respiratory virus testing includes either culture or respiratory PCR panel from respiratory specimens (eg, nasopharyngeal swab, nasal wash). Respiratory virus testing should include Influenza A and B (during influenza season). Testing for other respiratory viruses such as parainfluenza and adenovirus should be considered although their role in causing CNS illness is controversial.

Limited testing may be available through research laboratories, and includes examination of CSF or other affected tissues (ie, eye, muscle) for presence of parasite, or detection of antibody in serum or CSF.

Description

Relatively few causes account for the vast majority of identified cases of encephalitis [5, 7, 27]. Therefore, we recommend testing for these agents, along with selected, treatable conditions, in all individuals. Obtaining a comprehensive case history, including recent and remote travel, animal contacts and insect exposure, and carefully characterizing presenting symptoms, signs, and laboratory and neuroimaging findings are crucial to inform additional testing (Tables 2 and 3). We developed distinct algorithms for adult and pediatric populations, because the spectrum and frequencies of etiologies differ between the 2 age groups [27]. We recommend...
### Table 3. Diagnostic Algorithm for Initial Evaluation of Encephalitis in Children

<table>
<thead>
<tr>
<th>ROUTINE STUDIES</th>
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<tbody>
<tr>
<td><strong>CSF</strong></td>
<td>Collect at least 5 cc fluid, if possible; freeze unused fluid for additional testing</td>
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<tr>
<td>Opening pressure, WBC count with differential, RBC count, protein, glucose</td>
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<tr>
<td>Gram stain and bacterial culture</td>
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<tr>
<td>HSV-1/2 PCR (if test available, consider HSV CSF IgG and IgM in addition)</td>
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<tr>
<td>Enterovirus PCR</td>
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| **SERUM** |
| Routine blood cultures |
| EBV serology (VCA IgG and IgM and EBNA IgG) |
| **Mycoplasma pneumoniae** IgM and IgG |
| Hold acute serum and collect convalescent serum 10–14 d later for paired antibody testing |

| **IMAGING** |
| Neuroimaging (MRI preferred to CT, if available) |

| **NEUROPHYSIOLOGY** |
| EEG |

| **OTHER TISSUES/FLUIDS** |
| **Mycoplasma pneumoniae** PCR from throat sample |
| Enterovirus PCR and/or culture of throat and stool |

| **WHEN CLINICAL FEATURES OF EXTRA-CNS INVOLVEMENT ARE PRESENT, WE RECOMMEND ADDITIONAL TESTING (Eg, BIOPSY OF SKIN LESIONS; BRONCHOALVEOLAR LAVAGE AND/OR ENDOBRONCHIAL BIOPSY IN THOSE WITH PNEUMONIA/PULMONARY LESIONS; THROAT SWAB PCR/CULTURE IN THOSE WITH UPPER RESPIRATORY ILLNESS; STOOL CULTURE IN THOSE WITH DIARRHEA); ALSO SEE BELOW** |

| **CONDITIONAL STUDIES** |
| **HOST FACTORS** |
| Age <3 y—Parechovirus PCR (CSF) |
| Immunocompromised—CMV PCR, HHV6/7 PCR, HIV PCR (CSF); cryptococcal antigen; Toxoplasma gondii serology and/or PCR; MTB testing; fungal testing; WNV testing |

| **GEOGRAPHIC FACTORS** |
| Africa—malaria (blood smear); trypanosomiasis (blood/CSF smear, serology from serum and CSF); dengue testing |
| Asia—Japanese Encephalitis Virus testing; dengue testing; malaria (blood smear); Nipah virus testing (serology from serum and CSF; PCR, immunohistochemistry, and virus isolation in a BSL4 lab can also be used to substantiate diagnosis) |
| Australia—Murray Valley encephalitis virus testing; Kunjin virus testing; Australian Bat Lyssavirus (ABL) testing |
| Europe—Tick-borne Encephalitis Virus (serology); if Southern Europe, consider WNV testing, Toscana virus testing |
| Central and South America—dengue testing; malaria (blood smear) |
| North America—Geographically—appropriate arboviral testing (eg, WNV, Powassan, LaCrosse, Eastern Equine Encephalitis viruses, Lyme (serum ELISA and Western blot) |

| **SEASON AND EXPOSURE** |
| Summer/Fall: Arbovirus and tick-borne disease testing |
| Cat (particularly if with seizures, paucicellular CSF)—Bartonella antibody (serum), ophthalmologic evaluation |
| Tick exposure—Tick borne disease testing |
| Animal bite/bat exposure—rabies testing |
| Swimming or diving in warm freshwater or nasal/sinus irrigation—Naegleria fowleri (CSF wet mount and PCR) |

| **SPECIFIC SIGNS AND SYMPTOMS** |
| Abnormal behavior (eg, new onset temper tantrums, agitation, aggression), psychotic features, seizures or movement disorder—NMDAR antibody (serum, CSF), oligoclonal bands, IgG index, rabies testing |
| Behavior changes followed by myoclonic spasms/jerks: measles IgG (CSF and serum) |
| Vesicular rash—VZV PCR from CSF (sensitivity may be low; if test available, consider CSF IgG and IgM; VZV IgG and IgM from serum) |
| Rapid decompensation (particularly with animal bite history or prior travel to rabies-endemic areas)—rabies testing |
| Respiratory symptoms—chest imaging (chest X-ray and/or CT scan); respiratory virus testing; Mycoplasma pneumoniae PCR (CSF) |
| Acute flaccid paralysis—Arbovirus testing; rabies testing |
| Parkinsonism—Arbovirus testing; Toxoplasma serology |
| Nonhealing skin lesions—Balamuthia, Acanthamoeba testing |
| Prominent limbic symptoms—Autoimmune limbic encephalitis testing, HHV6/7 PCR (CSF) |
LABORATORY FEATURES

If EBV serology is suggestive of acute infection, perform EBV PCR (CSF)

Elevated transaminases—Rickettsia serology, tick borne diseases testing

CSF protein >100 mg/dL, or CSF glucose <2/3 peripheral glucose, or lymphocytic pleocytosis with subacute symptom onset—MTB testing, fungal testing, *Balamuthia mandrillaris* testing

CSF protein >100 mg/dL or CSF glucose <2/3 peripheral glucose and neutrophilic predominance with acute symptom onset and recent antibiotic use—CSF PCR for *S. pneumoniae* and *N. meningitidis*

CSF eosinophilia—MTB testing, fungal testing, *Baylisascaris procyonis* antibody (serum and CSF); *Angiostrongylus cantonensis*, *Gnathostoma* sp. testing

Hyponatremia—MTB testing

Mycoplasma pneumoniae* serology or throat PCR positive—Mycoplasma pneumoniae* PCR (CSF)

NEUROIMAGING FEATURES

Frontal lobe—*Naegleria fowleri* (CSF wet mount and PCR)*

Temporal lobe—HHV 6/7 PCR (CSF)

Basal ganglia and/or thalamus—Respiratory virus testing; Arbovirus testing; MTB testing

Brainstem—respiratory virus testing; Arbovirus testing; Listeria PCR (if available); Brucella antibody (serum); MTB testing

Cerebellum—VZV PCR from CSF (sensitivity may be low; if test available, consider CSF IgG and IgM; VZV IgG and IgM from serum; EBV PCR (CSF)

Diffuse cerebral edema—respiratory virus testing

Space occupying and/or ring-enhancing lesions—MTB testing; fungal testing; *Balamuthia mandrillaris* and *Acanthamoeba* testing

*Toxoplasma gondii* serology

Hydrocephalus and/or basilar meningeal enhancement—MTB testing; fungal testing; *Balamuthia mandrillaris* testing; Infarction or hemorrhage—MTB testing; fungal testing; respiratory virus testing

White matter lesions—Oligoclonal bands, IgG index, Lyme (serum ELISA and Western blot); Brucella (serology or CSF culture); Measles virus testing for SSPE; *Baylisascaris procyonis* antibody (serum and CSF); *Balamuthia mandrillaris* testing

Abbreviations: ABLV, Australian bat lyssavirus; BSL4, biosafety level 4; CNS, central nervous system; CMV, cytomegalovirus; CSF, cerebral spinal fluid; CT, computed tomography; EBV, Epstein-Barr virus; EBNA, Epstein-Barr virus nuclear antigen; EEG, electroencephalography; ELISA, enzyme-linked immunosorbent assay; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MRI, magnetic resonance imaging; MTB, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction; RBC, red blood cell; HSV, herpes simplex virus; RBC, red blood cell; NMDAR, N-methyl-D-aspartate receptor; VCA, viral capsid antigen; VDRL, Venereal Disease Research Laboratory; VGKC, voltage gated potassium channel; VZV, varicella-zoster virus; SSPE, subacute sclerosing panencephalitis; WBC, white blood cell; WNV, West Nile virus.

a This table is not intended to encompass all causes of encephalitis, nor all epidemiological or laboratory-based risk factors. We recommend utilizing this table as a guideline for initial management of acute encephalitis in children beyond the neonatal period. For additional information, we recommend consulting Tunkel et al. 2008, Steiner et al. 2010, Kneen et al. 2012 (see references). Consultation with local health authorities is also recommended.

b Although some members of the consortium recommended *M. pneumoniae* CSF PCR as routine testing for all children, a consensus was not reached given the challenges of establishing a diagnosis of encephalitis due to *M. pneumoniae* (see text).

c MTB testing includes CSF smear for acid-fast bacilli and CSF mycobacterial culture along with one or more of the number of MTB PCR tests for CSF now commercially available. Sensitivity of smear and culture increases with the volume of CSF analyzed; we recommend consulting with the laboratory regarding optimal volumes of CSF to be analyzed. Given the varying sensitivity of these tests, systemic MTB testing including tuberculin skin test (may be negative) or interferon gamma release assay, stains and cultures from sputum, and tissue from biopsies from any potential systemic sites of infection.

d Fungal testing should be tailored to specific geographic region and prior travel history/place of residence, and typically consists of serology, antibody testing from urine and/or CSF, and cultures from blood and CSF.

e Arbovirus testing can be performed for select arboviruses (ie, WNV, California serogroup viruses), and is particularly useful in immunocompromised patients.

f Babesia/ABLVL testing includes serologic analysis of serum and CSF; virus isolation or RT-PCR from saliva; tests for viral antigen or histopathology on either a brain biopsy or full-thickness biopsy of the nape of the neck. Testing should be conducted in concert with a local or regional public health department.

g Tick borne disease testing should be tailored to specific geographic region and typically consists of serology (ie, *Borreia, Ehrlichia, Rickettsia* spp., *Anaplasma phagocytophilum*, TBEV), and blood PCR (*Ehrlichia, Anaplasma*).

h *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Acanthamoeba* spp. testing is only available at specialized laboratories (eg, CDC) and includes serum immunofluorescence assay, immunohistochemistry on brain or other tissue and PCR testing on brain or other tissue and CSF. In addition, CSF wet mount is recommended for *Naegleria fowleri* testing. Brain tissue from affected region offers optimal sensitivity and specificity but other specimens can be tested.

i Respiratory virus testing includes either culture or respiratory PCR panel from respiratory specimens (eg, nasopharyngeal swab, nasal wash). Respiratory virus testing should include Influenza A and B (during influenza season). Testing for other respiratory viruses including *Paramyxoviridae* 1-4, Adenovirus, and human metapneumovirus should be considered although their role in causing CNS illness is controversial.

j Autoimmune limbic encephalitis evaluation includes testing for antibodies to VGKC, GAD, AMPA receptor, GABA* receptor, ngluRI, Hu, CV2, Ma2, and amphipysin.

k Limited testing may be available through research laboratories, and includes examination of CSF or other affected tissues (ie, eye, muscle) for presence of parasite, or detection of antibody in serum or CSF.
neuroimaging (preferably magnetic resonance imaging [MRI]), electroencephalography (EEG), and lumbar puncture (LP) in all individuals unless contraindicated [28], because such testing may confirm the diagnosis of encephalitis and establish the etiology.

If the etiology of encephalitis is not rapidly identified or where unique epidemiologic factors or clinical features are present, we recommend referring to several recent publications as a guide to further evaluation [1, 23–26]. Our recommendations incorporate large-scale geographic considerations; however, specific travel history or geographic information should prompt consultation with regional public health departments. Because our focus is on initial evaluation of patients, modalities such as brain biopsy, typically reserved for refractory cases of encephalitis, are not included. Moreover, our knowledge of autoimmune encephalitis is rapidly changing, with ongoing identification of novel autoantibodies and expansion of clinical spectra of disease. Here, we include well-recognized syndromes and relatively common etiologies [29, 30]. Overall, it should be noted that our recommendations provide general guidance for initial evaluation of encephalitis, but rapid advances in autoimmune encephalitis coupled with the emerging nature of infections warrant ongoing evaluation of testing paradigms.

**Selected Etiology-specific Considerations**

**Herpes Simplex Virus (HSV)**

Case series and studies have shown that HSV polymerase chain reaction (PCR) can be falsely negative, especially among children and early in the disease course [18, 21, 31]. If testing from the first LP is negative and herpes simplex encephalitis (HSE) is still of concern (eg, temporal lobe involvement seen on neuroimaging), a second LP should be repeated within 3–7 days with CSF sent for HSV PCR [1]. Testing for intrathecal HSV antibodies may complement molecular testing but is not typically useful for acute patient management [32].

**Varicella-zoster Virus (VZV)**

VZV is one of the most commonly identified causes of acute encephalitis in adults [5, 7], typically associated with viral reactivation and resulting in a CNS vasculopathy [33]. Notably, CNS reactivation may occur in the absence of skin lesions [34]. In children, on the other hand, most cases occur concurrently with chickenpox or in a post-infectious form [22, 35]. Detection of antibodies to VZV in the CSF appears to have greater sensitivity than detection of viral DNA [36]; therefore, we recommend that both assays be sent when possible.

**Enteroviruses (EV)**

CSF PCR analysis is crucial to perform but alone may be insufficient for diagnosis. In one report of an EV71 outbreak, EV-PCR of CSF yielded positive results in only 31% of cases, with higher yields from PCR of throat and stool specimens [37]. Because enteroviral shedding from the gastrointestinal tract may persist for weeks following infection [38], we recommend testing of both CNS and extra-CNS samples. Moreover, because standard EV PCR assays do not detect parovirus, specific PCR assays for these viruses should be performed in young children.

**Epstein-Barr Virus (EBV)**

EBV is an important cause of encephalitis in the pediatric population, particularly among adolescents. Although helpful in diagnosis of EBV-associated encephalitis, PCR testing can be associated with false-negative and false-positive results, the latter often occurring due to presence of EBV DNA in peripheral blood mononuclear cells. Therefore, serology, including antiviral capsid antigens (VCA) immunoglobulin M/immunoglobulin G (IgM/IgG) and anti-Epstein-Barr nuclear antigen (EBNA), is recommended in addition to CSF PCR [39].

**Human Herpesvirus 6 (HHV-6)**

The CNS pathogenic potential of HHV-6 has yet to be defined, although increasing evidence implicates its role in limbic encephalitis in the immunocompromised individual [40]. A positive HHV-6 CSF PCR should prompt corresponding evaluation of blood PCR levels in an effort to distinguish between chromosomal integration and acute infection [41]. Notably, latent disease can also be detected through PCR and may be a confounder [42].

**Arboviruses**

For most arboviruses, serologic testing of serum and CSF is preferred to molecular testing, since the peak of viremia typically occurs prior to symptom onset. For example, in patients with West Nile virus (WNV) associated with neuroinvasive disease, CSF PCR is relatively insensitive (57%) compared with detection of WNV IgM in CSF [43]. The cumulative percentage of seropositive patients increases by approximately 10% per day during the first week of illness, suggesting the need for repeat testing if the suspicion for disease is strong in those with initially negative results [44, 45]. Notably, arbovirus IgM antibodies may be persistently detectable in the serum and, less commonly, in the CSF, for many months after acute infection, and therefore may not be indicative of a current infection [46, 47]. Therefore, if possible, documentation of acute infection by seroconversion and/or 4-fold or greater rises in titre using paired sera is recommended.

**Mycoplasma pneumoniae**

Several reports have implicated *Mycoplasma pneumoniae* as a leading cause of encephalitis, particularly among children [48, 49]. In most such cases an immune-mediated mechanism is hypothesized; a preceding respiratory prodrome and detection of the pathogen in the respiratory tract, but not CSF, is typical of such cases. Direct infection of the brain or CSF is less common but has been observed in both adults and children. Serology alone
is unreliable in diagnosing neurologic disease due to *M. pneumoniae* because of the high background incidence of acute infections and limited specificity of currently available assays [50]. Similarly, because detection of *M. pneumoniae* DNA in respiratory secretions may reflect acute infection, remote infection or asymptomatic colonization its detection does not establish it as the cause of neurologic disease. We recommend that testing be performed in pediatric patients and include both serology and PCR analysis. Overall, the strength of microbiologic evidence needs to be considered when implicating *M. pneumoniae* as the cause of encephalitis [51].

**Anti-NMDA Receptor (NMDAR) Encephalitis**

Affected individuals typically develop prominent psychiatric symptoms, cognitive dysfunction, seizures, orofacial dyskinesias, and autonomic instability [52, 53]. Sensitivity of testing is approximately 15% higher from the CSF than from serum, as determined by comparison of paired serum and CSF samples [54]. Notably, the recent demonstration of serum or CSF antibodies to NMDAR in 30% of individuals during the course of typical HSE suggests that a positive antibody result should be interpreted in the proper clinical context [55].

**Autoimmune Limbic Encephalitis (ALE)**

ALE, characterized by rapidly progressive short-term memory deficits, psychiatric symptoms, and seizures, is associated with a wide variety of autoantibodies, including onconeural antibodies (ie, Hu, CV2, Ma2, amphiphysin) and antibodies to neuronal cell surface synaptic antigens (ie, voltage gated potassium channel [VGKC], glutamic acid decarboxylase, AMPA receptor, GABA<sub>B</sub> receptor, mgluR5). Although the former group is highly associated with underlying tumor, in the latter group the presence of malignancy is variable. In most cases, serum testing is sufficient [56].

**Summary**

This algorithm represents a practical tool for use by clinicians in initial evaluation of patients with suspected encephalitis and provides the basis for worldwide collaboration to advance diagnosis and management of affected individuals. To maximize the benefits of such an approach for research purposes, the use of standardized case history forms with relevant demographic and laboratory data is critical.

**PRIORITY 3: HOST GENETICS**

**Introduction**

Although encephalitis is typically a rare clinical entity, it follows infection with a number of relatively common agents. Reasons for this range of disease severity remain unclear. Several general and disease-specific risk modifiers have been identified, including infectious dose, viral or microbial genotypic variation, and age-related changes in anatomic barriers or global immune function. In addition, an individual’s genetic make-up contributes significantly to the variation in infectious disease susceptibility and severity [57]. Preclinical studies have identified host cell factors that modulate the course of infection for a range of microbes. With few exceptions, however, these studies have failed to identify genes in which human variation affects disease outcome. Indeed, risk alleles for infectious encephalitis have only been identified in a handful of cases (Table 4 and references).

**Challenges and Solutions**

The rarity and highly sporadic nature of encephalitis presents certain challenges. The strategy and approach to identifying genotypic determinants of a given phenotype depends largely on its allelic architecture—the number, type, penetrance, and frequency of disease associated variants (Supplementary Table 2) [58]. Mendelian traits, representing one extreme on the allelic spectrum, are determined by variants at a single locus. Because Mendelian variants are associated with a high relative risk of disease, they tend to be very rare in populations. Such traits have typically been dissected through linkage studies of families. While this approach has successfully identified genes involved in primary immunodeficiency, it is difficult to identify large pedigrees with multiple exposed and affected individuals for encephalitis and many other infectious diseases [57]. Genomewide resequencing of unrelated cases has emerged as another promising approach in Mendelian disease genetics. This strategy can identify candidate genes with as few as 10–50 individuals, but the case only design necessitates larger validation studies with appropriate controls [59, 60].

On the other end of the allelic spectrum are common genetic variants, which typically have only a modest effect on a disease phenotype. The common disease-common variant hypothesis predicts that many prevalent diseases are the result of common variants in multiple genetic loci, each with a small relative risk. These loci are typically identified in case-control association studies, although even the best candidate gene studies are prone to confounding and bias. While genomewide association studies circumvent many of these issues, adequate statistical power requires recruitment of hundreds or thousands of affected cases and exposed controls [61]. Even then, current study designs are poorly sensitive for rare alleles.

Given the large number of cases required and the cost of genomewide studies, human genetics has become a highly collaborative enterprise. However, many challenges exist in organizing such a genetics research effort. Above all, a multicenter approach will require a set of standard protocols for prospective subject recruitment, informed consent, biospecimen collection, and storage. While many investigators routinely collect serum
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\(^a\) Yakub et al., J Infect Dis 2005; 192:1741–48.  
\(^b\) Lim et al., PLoS Pathog 2009; 5:e1000321.  
\(^c\) Bigham et al., PLoS ONE 2011; 6:e24745.  
\(^f\) Lim et al., J Infect Dis 2010; 201:178–85.  
\(^g\) Casrouge et al. Science 2006; 314:308–12.  
\(^j\) Perez de Diego et al., Immunity 2010; 33:400–411.  
\(^k\) Sancho-Shimizu et al., J Clin Invest 2011; 121:4889–902.  
\(^m\) Herman et al., J Exp Med 2012; 209:2567–1582.  
and CSF from enrolled patients, protocols would need to be expanded to include snap-frozen whole blood with explicit authorization for future use in genetic studies. Similarly, common case history forms are needed to record demographics and relevant risk factors. Ideally, the biospecimens and clinical metadata would be curated and maintained in a central biobank with a mature informational technology infrastructure. The cost of such efforts would be significant.

The group also discussed how research efforts could impact the diagnosis and management of encephalitis. The rapid pace of gene discovery suggests a future in which genetic testing targets high-risk patients in need of immunization or those who would benefit from specific therapeutic interventions. This approach is now commonplace in oncology. In infectious disease, testing for HLA-B5701 is used to identify patients infected with human immunodeficiency virus (HIV) at risk for abacavir hypersensitivity [62], and IL-28B genotype may predict the clinical efficacy of interferon regimens for hepatitis C [63].

Summary

Overall, the identification of genetic risk factors for encephalitis and other neuroinvasive complications of infection is a priority research area. We expect that a more complete understanding of encephalitis host genetics will elucidate pathogenic mechanisms, define relevant biomarkers, and suggest potential therapeutic approaches. As is the case for clinical risk factors, genetic risk factors for encephalitis will likely include alleles that are pathogen-specific as well as mutations that confer broad susceptibility to encephalitis [64]. More work is clearly needed in this area, and this and other consortia can play a productive role in this movement to personalized medicine.

PRIORITY 4: SELECTED EMERGING AREAS

A discussion of selected emerging areas in encephalitis was held together with colleagues from the Centers for Disease Control and Prevention and Public Health Agency of Canada who attended our consortium meeting. Here, we identify priorities for the study of three pathogen groups: arboviruses, lyssaviruses (including rabies), and free living amoebae (FLA) (Table 5).

Arboviruses

Most arboviruses of medical importance belong to 3 families: Flaviviridae, Togaviridae, and Bunyaviridae [65, 66]. Japanese encephalitis virus (JEV) is the leading cause of mosquito-borne encephalitis globally and continues to expand its range, Tick-borne encephalitis virus (TBEV) is the most common arthropod transmitted viral infection of humans in Europe, and increasing numbers of cases of neuroinvasive disease have been documented in North America involving tick-transmitted Powassan virus [66–69]. West Nile virus (WNV) has re-emerged as an important cause of encephalitis in the United States and Europe, and there has been increasing recognition of dengue (the most common arboviral infection worldwide) and chikungunya viruses as causes of neurological complications [70–74]. La Crosse virus continues to be a leading cause of pediatric encephalitis in the United States, whereas detection of other members of the California serogroup causing severe disease may be hampered by a lack of commercially available diagnostic assays and low level surveillance [75, 76] (Table 5).

Lyssaviruses

Rabies, an acute progressive viral encephalitis with the highest case fatality known for any agent, is caused by viruses in the family Rhabdoviridae, genus Lyssavirus. Although rabies is one of the oldest infectious diseases, and efficacious human and animal vaccines were developed decades ago, the global public health and veterinary burden remains high. Tens of thousands of human deaths, and millions of exposures, occur annually, mostly in developing countries [77]. Reservoirs predominate among the Carnivora and Chiroptera (bats) [78, 79]. Outcome after exposure likely represents a continuum, defined in part by viral type, dose, route, and poorly understood host attributes [80, 81]. The reduction of exposure to rabid animals and postexposure prophylaxis after an animal bite are the 2 most relevant strategies to prevent additional human cases [82–84]. Elimination of canine rabies by mass vaccination, humane population management, and production of more effective, less costly biologics are solutions to reduce the burden [85, 86] (Table 5).

Free Living Ameboes (FLA)

Several FLA, including Naegleria fowleri, Balamuthia mandrillaris, and Acanthamoeba spp. cause CNS infections. N. fowleri causes primary amoebic meningoencephalitis (PAM), whereas B. mandrillaris and Acanthamoeba spp. generally cause a more chronic disease, granulomatous amebic encephalitis (GAE). Although case reports of PAM are rare, many additional cases likely go unrecognized, as suggested by the 75% of US PAM cases that are diagnosed postmortem (CDC unpublished data). Prior to 2010, PAM cases were reported only from southern US states. Recently, however, 4 cases were reported from Northern and Midwestern states (CDC unpublished data). Exposure to FLA is believed to be common; a recent serologic investigation showed 3–4% of individuals with evidence of B. mandrillaris exposure [87]. It remains unclear why some develop disease while the majority of those exposed do not [88]. B. mandrillaris GAE, previously only reported as isolated cases, has recently been diagnosed in multiple organ transplant recipients where the donor was found to have had B. mandrillaris infection, highlighting this organism as a potentially under-recognized cause of fatal encephalitis [89, 90]. Our knowledge of the contribution of FLA to human disease is limited by the lack of
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Abbreviations: CDC, Centers for Disease Control and Prevention; FLA, free living amoeba; GAE, granulomatous amoebic encephalitis; JEV, Japanese encephalitis virus; LACV, La Crosse Virus; PAM, primary amoebic meningoencephalitis; TBEV, Tick-borne encephalitis virus; WNV, West Nile virus.

<sup>a</sup> Kulkarni et al., Epidemiology and Infection 2012 (epub ahead of print).
<sup>b</sup> De Filette et al., Vet Res 2012; 43:16.
<sup>d</sup> Lee et al., J Gen Virol 2012; 93:20–26.
<sup>f</sup> Wieten et al., Clin Infect Dis 2013; 41:4–19.
<sup>g</sup> Jackson AC, Antiviral Res 2013 (epub ahead of print).
<sup>i</sup> Kim et al., Antimicrob Agents Chemother 2008; 52:4010–16.
consistent surveillance data and a restricted understanding of the ecology of FLA (Table 5).

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**References**


