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# Eastern Equine Encephalitis

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Eastern equine encephalitis (EEE) is a rare disease that can affect the central nervous system (CNS) and cause severe complications and death. The etiologic agent, eastern equine encephalitis virus (EEEV), is a highly pathogenic arbovirus endemic to North, Central and South America. EEEV is a member of the genus *Alphavirus*, family *Togaviridae* [1]. Other medically important alphaviruses found in the Americas include, Venezuelan equine encephalitis virus, chikungunya, Mayaro and Madariaga. EEEV is a spherical, enveloped virus that has a single-stranded, positive-sense RNA genome [2] that is 11,700 nucleotides in length [3].

EEEV is spread to horses and humans by infected mosquitoes [4]. Similar diseases are Western equine encephalitis, St. Louis encephalitis and LaCrosse encephalitis. The virus is also capable of infecting other mammals, birds, amphibians and reptiles [5]. The enzootic transmission of EEEV is maintained in a cycle between *Culiseta melanura* mosquitoes and avian hosts in freshwater hardwood swamps [4]. Since it feeds almost exclusively on birds, *Cs. melanura* is not considered to be an important vector of EEEV to humans. Transmission to humans requires mosquito species that bite both infected birds and uninfected mammals such as some *Aedes*, *Coquillettidia* and *Culex* species [6]. The level of viremia in the blood of humans and horses is largely insufficient to infect mosquitoes that bite them, so they do not serve as a source for ongoing spread of the disease; as such, both humans and horses are considered dead-end hosts for EEEV.

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# Eastern Equine Encephalitis (continued)

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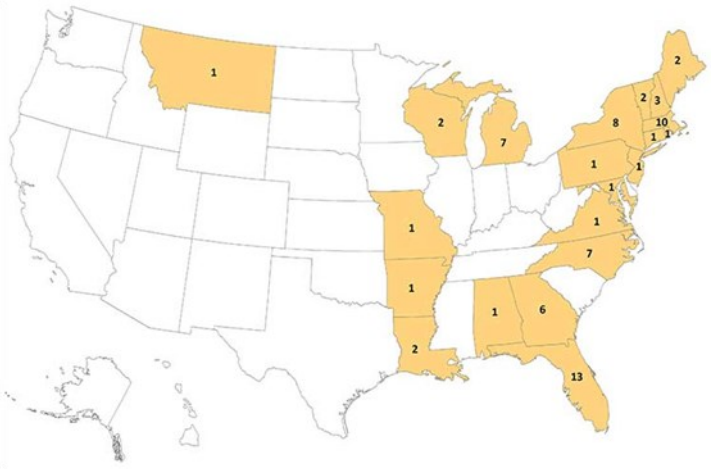
Although EEEV infection is asymptomatic in most birds, infected emus have been documented to develop a fatal bloody diarrhea and can transmit EEEV directly to humans who come in contact with infected blood or feces [7].

Organ transplantation has been demonstrated as an unusual mode of transmission of EEEV. In 2017, three solid organ transplant recipients developed encephalitis within one week of transplantation. The three organs originated from a common donor and prompted suspicion of transplant-transmitted infection. Analysis of endomyocardial tissue from the heart recipient identified EEEV [8].

The incubation period for EEEV disease ranges from 4 to 10 days. Most arboviral infections are asymptomatic. If symptomatic, clinical disease ranges from mild febrile illness to severe encephalitis or coma and lasts 1 to 2 weeks. People under the age of 15 or over 50 seem to be at greatest risk for severe disease.

The mortality rate for symptomatic neuroinvasive cases of EEE is 35% or more. Death usually occurs 2 to 10 days after onset of symptoms but can occur much later. Survivors of neuroinvasive disease often face disability from neurological sequelae [9], which can range from mild brain dysfunction to severe intellectual impairment, personality disorders, seizures, paralysis and cranial nerve dysfunction. Many patients with severe sequelae require long-term care and die within a few years. Non-neuroinvasive symptoms includes headache, myalgias, arthralgia, rash, vomiting or diarrhea.

Figure 1. EEEV neuroinvasive disease cases reported by state of residence, 2009–2018.




Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

EEE was first recognized in humans in 1938. In the United States, EEEV transmission occurs in the Northeast, Southeast and Midwest, but the largest number of horse and human cases have been reported in Florida. Annually, an average of one or two human cases and over 60 equine cases of EEE are reported in Florida, with most activity occurring in the Panhandle [10]. Between 2009 and 2018, 72 human cases of EEE were reported in 21 U.S. states [11], with Florida accounting for 18% of all reported human cases (Figure 1). Thirty (42%) fatalities occurred in this time period. Florida is the only state where transmission occurs year-round [12], although most activity is seen between May and August. For other affected states, a study in 2012

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indicated that snakes may harbor EEEV through winter hibernation which could allow transmission of the virus to the next season [13]. However, a whole genome sequencing study published in 2018 found that Florida was most likely the source of EEEV to northern states [14].

There is no approved vaccine for humans or specific antiviral treatment for EEEV infections. Current methods consist primarily of symptom treatment and supportive care. A vaccine has been developed and is in use for horses, as the case-fatality in equines is 80-90%. The vaccine has also been used off-label for ratites (ostriches and emus) and camelids (alpacas and llamas).

The most effective way to prevent infection from EEEV is to prevent mosquito bites. Mosquito activity in Florida can be year-round. Local government work to reduce mosquito populations as part of an integrated mosquito management program.

Personal preventative measures include the following:

- Drain or eliminate places where the mosquito lays her eggs, primarily artificial containers that hold water.
- When outside, wear shoes, socks, long pants and long sleeves.
- Use screens on windows and doors. Repair holes in screens.
- Use Environmental Protection Agency-registered insect repellents:
  - DEET
  - Picaridin
  - IR3535
  - Oil of lemon eucalyptus (OLE) – Not for children under 3 years old
  - Para-menthane-diol (PMD) – Not for children under 3 years old
  - 2-undecanone
- Use permethrin-treated clothing and gear (such as boots, pants, socks and tents).
- Dress your child in clothing that covers arms and legs.
- Cover strollers and baby carriers with mosquito netting.

## Surveillance

Surveillance provides temporal and geographic information used to predict the likelihood of arboviral transmission to humans. This information directs the implementation of vector and disease control activities prior to and during an epidemic [15]. In Florida, environmental surveillance for EEEV includes serologic testing of sentinel chickens and direct detection of the virus in mosquitoes. For serologic testing of sentinel chickens, local county health departments and mosquito control agencies maintain the sentinel chicken flocks and are responsible for collecting and shipping

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blood specimens for arboviral laboratory diagnostic testing at the Bureau of Public Health Laboratories (BPHL) in Tampa.

Currently in Florida, there are 30 Mosquito Control Districts in 28 counties. Sentinel flocks are located in a variety of habitats throughout the state and include hardwood hammocks, pine flatwoods, coastal habitats, freshwater marshes, saltwater marshes, residential areas, city and county parks and urban centers. For direct testing of mosquitoes, a variety of traps are used to collect flying mosquitoes in places where arboviral transmission is suspected to be ongoing, and also includes ground aspirator collections at mosquito daytime resting sites, avian roosts and areas of past virus activity.

## Biosafety

Although not the natural route of transmission, alphaviruses are highly infectious by the aerosol route and laboratory acquired infections have been documented [16]. More than 160 laboratory-acquired infections with EEEV, Venezuelan equine encephalitis virus or Western equine encephalitis virus have been documented. Many infections were due to procedures involving high concentrations of virus and aerosol-generating activities such as centrifugation. Procedures involving animals and mosquitoes are also particularly hazardous.

EEEV may be present in blood, cerebrospinal fluid (CSF) and other tissues, such as brain. Primary laboratory hazards include aerosol inhalation, parenteral inoculation, contact with broken skin or mucus membranes and bites from infected animals or arthropods [17]. Diagnostic and research activities involving clinical material, infectious cultures and infected animals or arthropods should be performed under biosafety level 3 (BSL-3) practices, containment equipment and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered. Animal work with EEEV should be performed under Animal BSL-3 (ABSL-3) conditions.

## Laboratory Diagnostics

Laboratory diagnosis of arboviral infections is usually achieved by testing serum or CSF to detect virus-specific IgM. An IgM capture Enzyme-Linked Immunosorbent Assay (ELISA) is typically the test performed in commercial and sentinel clinical laboratories. Due to the potential for false-positive test results, all specimens testing positive at private laboratories must be forwarded to the state public health laboratory for confirmation. This also includes reactive specimens from blood bank donors.

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State public health laboratories have the capability to perform additional analyses and confirm infection. Both BPHL-Jacksonville and BPHL-Tampa perform the IgM capture ELISA on human serum and CSF, and IgG enzyme immunoassay on human serum only. In addition, BPHL-Tampa performs more extensive testing for EEEV. All specimens with a positive serology result are reflexed to the plaque-reduction neutralization test (PRNT). In PRNT, the serum or spinal fluid is first incubated with live EEEV and then added to a cell culture/agar mixture. If neutralizing antibodies to the virus are present in the specimen, they will bind to the live virus and prevent it from infecting the cell culture lawn. Thus, there will be a reduction in the number of plaques exhibiting a cytopathic effect when compared with a control culture where no neutralizing antibody has been added.

Regardless of the ELISA result, some high priority patients are tested by PRNT based on the epidemiological data from the case investigation. Additionally, specimens from exotic birds and other animals, such as dolphins and manatees, can be tested with PRNT. These are most often submitted by zoos or wildlife sanctuaries, or may be part of a research study.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) is also performed at BPHL-Tampa for direct detection of EEEV. The most common human specimen types are serum and CSF. Autopsy specimens, such as brain tissue, can be tested by RT-PCR, but thankfully this is a rare request. RT-PCR is also performed on animal tissue, such as horse brain, when EEEV is suspected as the cause of death. Additionally, BPHL-Tampa analyzes mosquito pools by RT-PCR.

In the past, many large sentinel clinical laboratories performed virus isolation in cell lines as part of their routine virology services. However, with the advent of less expensive and more sensitive rapid molecular analyses very few clinical laboratories maintain this methodology and its associated skillset in their technologists. BPHL-Tampa retains this diagnostic capability. For EEEV detection, both animal tissues and a subset of mosquito pools that test negative by RT-PCR are submitted for virus isolation. Virus isolation may be performed on human specimens under exceptional circumstances and is approved on a case-by-case basis. Histopathology with immunohistochemistry (IHC) specific for arboviruses is another laboratory diagnostic option, particularly for fatal cases. However, IHC is not available at BPHL and specimens would be referred to the Centers for Disease Control and Prevention (CDC).

## Sentinel Chicken Testing

Serum specimens from the sentinel chicken surveillance program are initially

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screened using a hemagglutination inhibition (HAI) test. HAI will detect antibodies to both alphavirus (EEEV and Highlands J virus) and flavivirus (West Nile virus and St. Louis encephalitis virus), but cannot differentiate between the genera. A negative HAI result will be reported as final. All other results are reflexed to the IgM capture ELISA assay for West Nile and St. Louis encephalitis viruses. A positive result will be reported as the respective virus antibody identification. Serum with negative, equivocal or inconclusive results are submitted for PRNT to identify antibodies to the specific virus that caused the reactive HAI result. EEEV infection in the sentinel chicken is identified at this point.

## Select Agent Aspects

Due to its potential to be used as biological weapon, EEEV is classified as a select agent under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and is regulated under 42 CFR Part 73. The Federal Select Agent Program (FSAP) is jointly managed and enforced by the CDC Division of Select Agents and Toxins and the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS). When identification of EEEV is confirmed from a clinical sample, FSAP must be notified and APHIS/CDC Form 4A completed. If a potential exposure occurred, APHIS/CDC Form 3 must also be completed [18]. In addition, all cases of suspected and confirmed EEE must be reported to the CDC's National Notifiable Diseases Surveillance System via the state health department.

Introduction of these stringent reporting requirements of the select agent regulations has heightened awareness of possible laboratory exposures and the required medical evaluation of laboratory personnel.

According to the regulations, certain strains of select agents that are less potent may be excluded from the legal requirements based upon a determination that the strain does not pose a severe threat to public and animal health and safety. The language in the existing regulations states that South American genotypes of EEEV

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fall into this exclusion and are not regulated as select agents. However, in more recent years, the South American strains were determined to be genetically distinct from EEEV and were reclassified as Madariaga [19]. Since our current EEEV RT-PCR test is not known to cross react with Madariaga, all strains of identified EEEV are reported to FSAP.

As a relatively rare zoonotic infection, EEEV presents several challenges in laboratory diagnosis and surveillance. For primary health care settings, laboratory diagnosis may not be feasible from a cost standpoint and proficiency to perform the necessary testing. Testing beyond EEEV serology may also not be possible, because of the select agent regulations. EEEV testing is available at the Florida BPHL upon request through county health departments.

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## Chemical Threat (CT) Preparedness Training

The Chemical Threat (CT) laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical threat awareness and the collection of clinical specimens after a chemical exposure event. Hospital and CHD staff play an important role in the response to a chemical exposure event when clinical specimens are collected for analysis. This is a one-hour course that covers both chemical agent awareness and post-exposure clinical specimen collection and shipping. This training is provided at no-cost and can be presented at your facility for your convenience. Training manuals and "hands-on" exercise materials will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel and others who may collect clinical specimens. Contact Angela Ren at (813) 223-2293 (Angela.Ren@FLHealth.gov) or Michelle Latona at (904) 791-1525 (Michelle.Latona@FLHealth.gov) for more information.

## Laboratory Response Network (LRN) Training—Biological Defense

The Bureau of Public Health Laboratories is currently offering an LRN sentinel laboratory training course at your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided as well as the biodefense reference manuals for your laboratory.

The training syllabus includes: an overview of the LRN; biosafety risk assessment and biosafety for the clinical laboratory; the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health Reference Laboratory when a bioterrorism agent cannot be ruled out; and an introduction to the CDC Select Agent Program.

To schedule a class contact:

*Rachel Clark at (813) 233-2367 (Rachel.Clark@FLHealth.gov)*

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