# TESTING METHODS FOR EASTERN EQUINE ENCEPHALITIS

Eastern Equine Encephalitis Virus (EEEV) is typically a rare disease, with only 5-15 cases on average being reported during 2010-2018. However, there was a significant increase in the number of human cases in 2019 and veterinary cases continue to be detected in jurisdictions where EEEV was not previously found. It is important for public health laboratories to ensure that access to appropriate testing for EEEV is available.

EASTERN EQUINE ENCEPHALITIS OVERVIEW

Eastern Equine Encephalitis (EEE) is a potentially fatal vector-borne disease that is caused by the alphavirus, eastern equine encephalitis virus (EEEV). It is transmitted between infected *Culiseta melanura* and *Culiseta morsitans* species of mosquitoes and birds. Human and animal cases usually result from other species of mosquitoes, such as *Aedes vexans*, *Coquillettidia perturbans*, *Ochlerotatus canadensis*, and *Ochlerotatus sollicitans*, serving as bridge vectors from infected birds.

EEEV is prevalent in several states across the Northeast, Upper Midwest and Southern states in the United States. While EEE most often affects hooved animals such as deer, sheep and horses, the number of human cases in the US increased significantly in 2019. The geographic span of the disease has also increased over the last five years and some states with no history of EEEV infection are now seeing cases emerge. Therefore, it is important that states consider taking action to assess EEEV cases in their own jurisdictions.

Figure 1: Eastern Equine Encephalitis cases reported by state, 2010-2019[a,b,c]

Legend:
- Human cases of EEEV 2010-2019
- Veterinary cases of EEEV 2010-2019
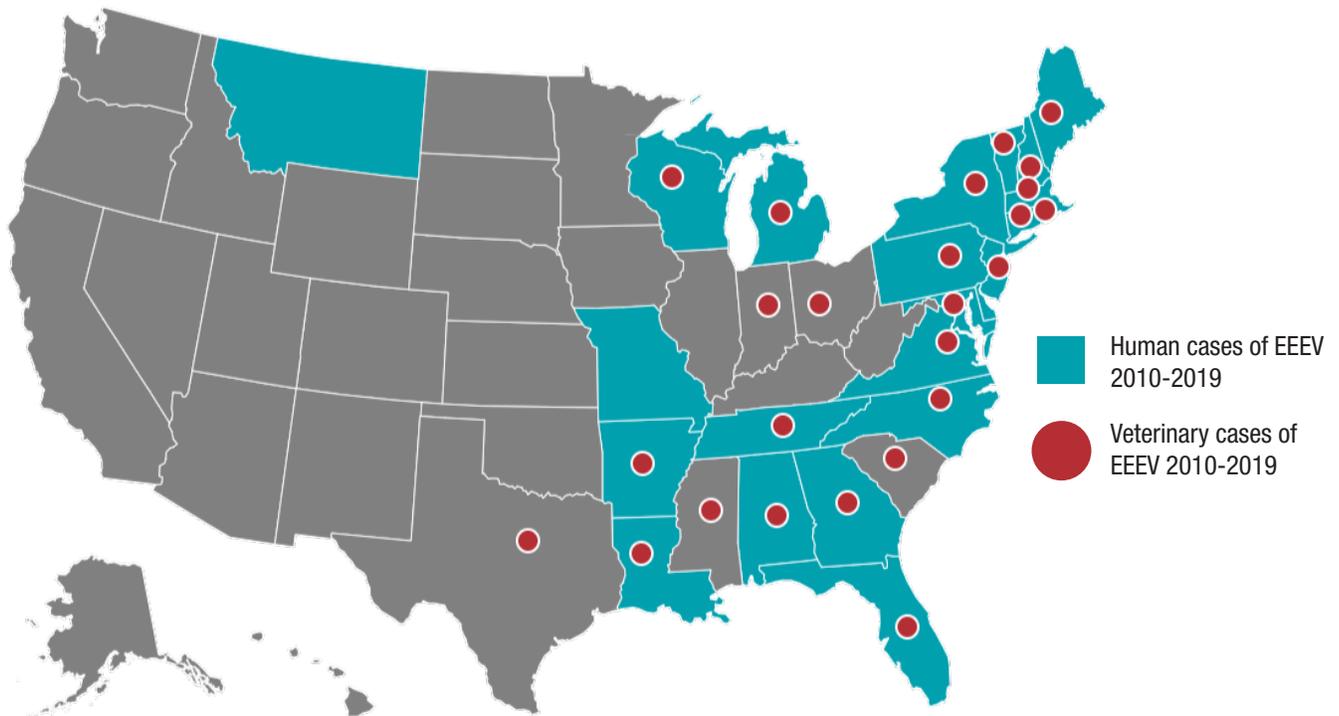
a. Centers for Disease Control and Prevention, ArboNET. Eastern Equine Encephalitis Virus Human and Veterinary Cases of EEE. Last Updated January 11, 2022, Available at https://wwwn.cdc.gov/arbonet/maps/ADB_Diseases_Map/index.html
b. Cases are reported based on patients' state of residence and therefore the represented data could include travel related EEEV cases
c. Data reported to CDC's ArboNet are reliant on voluntary reporting by states. State driven reporting can vary based on their capacity for EEEV surveillance

APHL
ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

TESTING METHODS

Test methods available for EEEV include serology and molecular based detection. Virus isolation can also be used to detect EEEV, but it is not a common practice as often times the viremic period of EEEV has passed prior to symptom onset, making it difficult to isolate the virus. Human and animal diagnoses via these testing methods typically require specific sample types such as serum, cerebrospinal fluid (CSF) or brain biopsies.

Serology Tests

Serologic assays that specifically detect IgM antibodies are the most commonly used testing methods for diagnosis of EEEV in clinical samples.

The IgM capture enzyme-linked immunosorbent assay (ELISA) technique is the most commonly used serologic assay for EEEV diagnosis among public health laboratories currently offering EEEV testing. Available commercial products to perform IgM capture ELISA have made the testing process fairly automated and interpretation of the results straightforward. Common sample types for the IgM capture ELISA include serum or CSF.

CDC has developed an IgM assay for EEEV detection—the EEEV IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA)—and can provide reagents and resources for PHLs interested in implementing the assay in their own laboratories. The microsphere-based immunoassay (MIA) is used for the clinical diagnosis of EEEV. For MIA, as with the IgM ELISA, serum and CSF are all acceptable sample types for the assay. Though similar in technique to the IgM capture ELISA, the MIA can be completed within one day, while most ELISA assays require two or three days to complete.

The IgG capture ELISA is not typically used for diagnostic testing and is primarily used in surveillance studies. As with other serologic assays, the IgG ELISA can be performed using serum and CSF.

Another assay for EEEV diagnosis is the Indirect Immunofluorescent Antibody (IFA) assay. Although this assay is performed in many PHLs, specialized knowledge in reading and interpreting the results is required, as the results are not quantitative and the interpretation can be subjective. EEEV specific commercial IFA testing kits and reagents are available for this assay and serum is the most commonly used sample type for this assay.

Most serologic assays work best when the samples are taken within 1-3 weeks from symptom onset (acute-phase). A sample that is antibody positive for EEEV via one of the aforementioned serologic assays should be confirmed with a plaque reduction neutralization test (PRNT).

Plaque Reduction Neutralization Test

Plaque Reduction Neutralization Test (PRNT) is used to confirm antibody positive EEEV samples. PRNT detects virus-specific neutralizing antibodies and can be used to confirm the presence of EEEV antibodies in both human and animal samples. Acute- and convalescent-phase serum specimens may be needed to measure a four-fold or greater rise in neutralizing antibody to confirm a recent infection or distinguish EEEV specific neutralizing antibodies from neutralizing antibodies of other alphaviruses.

Molecular Tests

Molecular testing methods, such as RT-PCR are available for diagnostic testing of EEEV. However, the viremic period for EEEV is very short, which reduces the testing window for this assay. Often molecular methods are used for post-mortem EEEV diagnosis in patients who rapidly decline. Brain tissue would be the best specimen type to confirm EEEV diagnosis in such cases.

Additionally, these assays can be used to test animal specimens. Similar to post-mortem human cases, animal brain tissue can be tested using these methods to determine presence of the virus in veterinary cases.

For the purposes of environmental surveillance, mosquito pools are tested via molecular based detection methods for the presence of EEEV. CDC has developed RT-PCR assays for the clinical and environmental diagnosis of EEEV, which PHLs can implement within their own laboratories.

ADDITIONAL RESOURCES

- Public health laboratories wishing to implement EEEV testing are encouraged to consult the *Biosafety in Microbiological & Biomedical Laboratories* guidance document, perform their own risk assessment and review the Federal Select Agent Program's select agent resources prior to implementation.

- CDC is able to provide diagnostic testing for EEEV. Public health laboratories wishing to submit samples that are suspected EEEV-positive can refer to the instructions for submitting diagnostic specimens to the Division of Vector-Borne Diseases' Arbovirus Diagnostic Testing Laboratory for testing and/or confirmation guidance.

- CDC has developed an RT-PCR assay for EEEV RNA and is able to provide resources, controls and protocols should public health laboratories wish to run molecular tests for EEEV in their laboratories. Since EEEV is a select agent, positive controls provided by CDC for the RT-PCR assay will be chimeric in nature in order to abide by select agent distribution restrictions. Reagents and controls are also available for PRNT. Public health laboratories can consult the DVBD Laboratory Resources website.

- APHL and CDC have developed an arbovirus IgM MAC-ELISA training. The training contains resources for implementing, conducting and interpreting the results of the MAC-ELISA for public health laboratories.

- Laboratories that do not have in-house testing for EEEV, or specialized confirmatory testing, such as PRNT, could inquire about testing availability in neighboring states via regional collaborations.