



Eastern Equine Encephalitis (EEE)

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Epidemiology

Eastern Equine Encephalitis (EEE) virus is an alphavirus belonging to the *Togaviridae* family, occurring in the eastern half of the United States. Other alphaviruses that can cause human infection include Western and Venezuelan equine encephalitis viruses. All of these viruses are transmitted to humans through the bite of an infected mosquito.

Like the West Nile (WNV) and St. Louis (SLE) viruses, the natural reservoir for the virus is birds and the virus is transmitted from bird to bird through mosquito bites. EEE surveillance in Louisiana includes the collection of mosquito pools and the use of sentinel chickens. Since a large number of horse cases and deaths usually precedes the identification of a human case, veterinarians are asked to report any horses exhibiting suspicious signs consistent with EEE to the State Veterinarian's Office and to submit serum for testing.

The most important mosquito in maintaining the EEE enzootic between birds is *Culiseta melanura*, this often occurs in coastal areas and freshwater swamps. Since this species rarely bites humans, *Cs. inoronata*, *Cx. quinquefasciatus* (the Southern House Mosquito), *Ae. albopictus* (the Asian Tiger mosquito), *Ae. sollicitans* (the tan salt marsh mosquito), *Ae. vexans* and *Coquillettidia perturbans*, are more important in the transmission of the virus to humans and horses.

In Louisiana, *Culex quinquefasciatus*, the Southern House Mosquito, is also the main vector of WNV and SLE. The females lay single raft of 150-350 eggs on heavily polluted small water collection after each blood meal. The eggs hatch in 1-2 days and become adults in 8-12 days. Preferred breeding places include; collections of ground water, storm sewer catch basin, ground pools, ditches, run off from sewage plants, small or large artificial containers, cesspits, drains, septic tanks, unused wells, and storm water canals. The flying range of adult female *Culex* is limited, up to 3,600 feet (1,200m) /night. They prefer feeding on birds and poultry, however, they also readily bite humans. They are most active at dusk and dawn but usually bite humans towards the middle of the night both indoors and outdoors.

Nationally, only about five cases are reported annually. Florida, Georgia, Massachusetts and New Jersey have reported the largest number of human cases. In Louisiana, the last outbreak occurred in 1999 with 2 human cases from Tangipahoa and Assumption parishes, 99 EEE+ horses, mostly from the southern half of the state, and more than 200 farm-raised emus. The most recent case was identified in 2003, also from Tangipahoa parish, a year that also had a great deal of horse activity documented along the U.S. Eastern seaboard.

Residents and visitors to endemic areas (with established presence of the virus) and those that engage in outdoor work and recreational activities are at greatest risk

An annual vaccine is available for horses, there is no licensed vaccine for human use.

Clinical Description

The incubation period for EEE is relatively short, 1 to 4 days.

About 25% of infections are symptomatic. Symptoms range from mild flu-like illness to encephalitis and coma. Persons over 50 or under 15 years are at greatest risk for developing severe disease. An estimated 35% of survivors will have mild to severe neurologic deficits.

The case fatality rate may exceed 50%, (range: 35-80%), the highest mortality rate of the domestic arboviral encephalitis. Clinical progression is relatively quick, with death occurring within 2-3 days after onset of symptoms.

Surveillance

All EEE infections are reportable conditions.

Report and Confirm Early Cases

Patients presenting with the following clinical syndromes should be suspected of having EEE illness particularly during the transmission season (May to November).

(1) Viral encephalitis, characterized by:

- Fever ≥ 38 °C or 100 °F, and
- CNS involvement, including altered mental status (altered level of consciousness, confusion, agitation, or lethargy) or other cortical signs (cranial nerve palsies, paresis or paralysis, parkinsonian signs, tremors, ataxia or convulsions), and
- An abnormal CSF profile suggesting a viral etiology (a negative bacterial stain and culture with pleocytosis [WBC between 5 and 1500 cells/mm³] and/or elevated protein level [≥ 40 mg/dl]).

(2) Aseptic meningitis (among persons aged 12 years and up), characterized by:

- Fever ≥ 38 °C or 100 °F, and
- Headache, stiff neck and/or other meningeal signs, and
- An abnormal CSF profile suggesting a viral etiology (a negative bacterial stain and culture with pleocytosis [WBC between 5 and 1500 cells/mm³] and/or elevated protein level [≥ 40 mg/dl]).

Indications for testing

Testing for arboviruses at the State Public Health Laboratory is being prioritized for hospitalized patients with viral encephalitis or aseptic meningitis. In order to keep the number of lab tests manageable, avoid testing asymptomatic patients bitten by mosquitoes, and the worried well.

There is no charge for arboviral encephalitis testing.

Specimens to obtain:

Acute phase (collected within 8 days of illness onset): 2 mL serum in labeled red top tube and CSF (if collected): 2 mL without preservatives

Convalescent phase (collected within 14-21 days of illness onset) At least 2 mL serum in labeled red top tube

Specimen labeling, packaging and mailing

-Label: patient's name, date of birth, medical record number, and date of specimen collection

All specimens should be accompanied by the appropriate form: "*Lab submission form for Arboviral Testing in Humans*"

Unless there is an emergency, avoid sending samples over the weekend or on holidays. Hold the samples for delivery until the next business day. In case of emergency, make prior arrangements with the laboratory (Virology Section 504-568-4039 or Infectious Disease Epidemiology Section 504-568-5005).

-Storage:

- **CSF:** Keep specimens refrigerated. Do not send or store at room temperature.
- **Sera:** Centrifuge, separate from clots, dispense into two sterile tubes (at least 2 cc each) for transport, and refrigerate (do not freeze).

-Packaging: Package CSF and sera in separate bags for transport to OPH. Pack blue ice or other coolants along with serum sample. Do not freeze. Do not use dry ice.

Ship to the following address:

Reporting test results:

All arboviral testing results will be faxed to the submitter (physician, hospital laboratories) by OPH lab.

Laboratory Tests

Test Methods

Testing is also performed for Eastern Equine Encephalitis (EEE), Saint Louis Encephalitis (SLE), and California Virus encephalitis using an **immunofluorescence technique (IFA)**.

If the IFA is positive at the State Lab, the sample will be forwarded to the Centers for Disease Control (CDC) for confirmatory testing. This testing will include, a **Plaque Reduction Neutralization Test (PRNT)**. The serum of the suspect is incubated with the live EEE virus then added to a cell culture. If there are antibodies against the virus in the test serum, there is reduction in virus damage compared to control with no antibodies (hence the term “Plaque Reduction”). This requires handling cell cultures and live virus and it may take several days to a week to evaluate the result.

Make sure to differentiate from IgG and IgM

IgG antibodies from a viral infection will last for years, even a lifetime. Therefore interpretation of an IgG positive test with an IgM negative result is consistent with an old infection and is not usually useful for the diagnosis of a recent clinical infection.

IgM antibodies do not cross the blood brain barrier therefore IgM antibodies in CSF strongly suggest central nervous system involvement. **IgM in blood may persist**, therefore there must be a strong clinical correlation for onset of symptoms consistent with the laboratory evidence. Convalescent blood samples should be drawn; a four-fold increase in the IFA IgM titer for EEE would further support the recognition of a current infection.

Fatal Encephalitis Cases

Fatal viral encephalitis cases of unknown etiology must be reported to the Infectious Disease Epidemiology Section (IDES).

- At least 5 cc of whole blood and 5 cc of serum may be frozen and held until decisions are made as to what specimens and tests are needed for further testing.
- Freezing at or below -20 degrees C is sufficient for short-term storage.

For specimen collection questions: During business hours, please call 504-568-5005 and ask to speak the Arboviral Surveillance Coordinator. After hours, call (800) 256-2748 or 504-568-5005 and request to speak to the epidemiologist on call.

EEE Encephalitis Surveillance in Sentinel Chickens and Mosquitoes

1. Sentinel Chickens

- Location of sentinel chickens must be based on entomological data to maximize benefits. Sentinel chickens may be used throughout the year.
- Chickens should be at least 9 weeks old. Maintain a supply of chickens in a mosquito free environment to replace infected chickens.
- Detection of antibodies by serologic tests is the method of choice.
- Chickens should preferably be bled every week.
- Chickens should be replaced after sero-conversion (positive for antibodies to any other arboviruses).
- Test method:

- IgM capture ELISA for Eastern equine encephalitis (EEE), St. Louis encephalitis (SLE), and West Nile virus (WNV).
- Plaque Reduction Neutralization Test (PRNT) will be performed when necessary.
- Lab tests done at the LSU Veterinary Lab. Results should be available within 5 days of sample receipt at the lab.

2. Mosquito Pools

- Pools should consist of 5-50 mosquitoes of the same species, some should be sorted to genus only.
- Test method:
 - VecTest (Medical Analysis Systems, Inc.) for *Culex quinquefasciatus* during the peak transmission season. The test can be done as a field test by the Mosquito Control Programs after training. The test is very specific (no false positives). Its sensitivity is similar to EIA tests. Results are obtained in 15 min. The test can be safely performed in the Mosquito Control District laboratories: Female mosquitoes are placed in a plastic culture tube, then a 2.5 mL of grinding solution is added which inactivates the virus and makes the rest of the test safe.
 - Reverse transcriptase - polymerase chain reaction (RT-PCR) TaqMan for all other species of mosquitoes and outside the peak transmission season. Lab tests done at the LSU Veterinary Lab. Results should be available within 5 days of sample receipt at the lab. (Currently only PCR for WNV is performed, in 2005 EEE and SLE will be added.)