July 10, 2014

RE: Availability of Eastern Equine Encephalitis and West Nile virus testing

Dear Vermont Veterinarian:

In 2013, two horses in Franklin County were diagnosed with Eastern equine encephalitis, and one horse in Lamoille County was diagnosed with West Nile virus infection. EEE virus was first detected in Vermont in 2011 when several emus in a flock died from the disease. In 2012 two horses were diagnosed in southern Addison and northern Rutland Counties. In addition, results of serosurveys from 2010 through 2012 showed widespread exposure to EEE virus in the deer and moose populations, so it is possible that EEE virus will cause illness in other parts of the state.

West Nile virus has been present in the state since 2000. It has been found in birds, mosquitoes, domestic animals or people in all counties. There does not appear to be any part of the state that is more at risk than others.

Although EEEV is an uncommon cause of human illness, it can be a devastating disease. The mortality rate approaches 35%, and people who survive often have persistent neurologic deficits. While WNV infection tends to result in less severe illness, it can be fatal and people can be left with permanent impairments. Both diseases are usually severe in unvaccinated susceptible animals. It is recommended that susceptible animals be vaccinated, and that people take precautions to prevent mosquito bites.

This summer, the Vermont Department of Health and the Vermont Agency of Agriculture, Food & Markets will again be offering testing of highly susceptible species, free of charge, from July 1st through November 15th. Specimens that meet the criteria for testing will be tested for both EEE virus and WNV.

About EEE virus in animals
EEE virus is a mosquito-borne viral disease that causes a progressive neurologic condition in horses and other equids. Alpacas, llamas and emus are also known to be susceptible to illness. The mortality rate in horses is 75 to 90 percent. Clinical signs of EEEV in horses include fever, depression, loss of appetite, weakness, ataxia, chewing movements, head pressing, circling, “sawhorse” stance, paddling, seizures, irritability, excitability, blindness, and abnormal sensitivity to light and sound. However, the illness in horses can also be peracute, and some die suddenly without showing obvious signs or symptoms. The signs and symptoms are not unique to EEEV. Other conditions to consider include West Nile virus encephalitis, tetanus, rabies, equine herpesvirus-1, equine protozoal myeloencephalitis, and western or Venezuelan equine encephalitis.
In emus, infection typically results in a rapid onset of clinical signs, often resulting in death. Common manifestations include disseminated intravascular coagulation, severe hemorrhagic enterocolitis, and blood-tinged vomitus. It is interesting to note that emus develop high levels of viremia. Unlike horses and humans, they may not be dead-end hosts and may act as a reservoir for the virus. There is evidence that the vaccines available for horses may protect emus and alpacas from infection with EEEV.\(^1\)\(^2\)

**Criteria for testing through the Vermont Department of Health Laboratory:**

Onset of illness: July 1\(^{st}\) through October 31\(^{st}\) (unless relevant travel history)

Species:
- Equids
- Camelids (ie. alpacas, llamas)
- Ratites (ie. emus)

Clinical illness:
- One or more of the following –
  - Ataxia or stumbling and incoordination
  - Inability to stand
  - Acute paralysis or limb weakness
  - Sudden death with no other diagnosis
  - Severe hemorrhagic enteritis (emus)

We will ask you to provide the following information:
- Date of onset of illness
- Date of specimen collection
- Address where animal is stabled/housed
- Vaccination history
- Travel history
- Disease history
- List of neurologic symptoms

Note: All brain tissue samples from equids and camelids that test negative for rabies at the Vermont Department of Health Laboratory will automatically be sent for arboviral testing.

**Sample submission:**

**Animals that died or were euthanized:**
- Brain tissue is the preferred sample, especially for non-equids and vaccinated animals. Most of these animals will have to be tested for rabies first. Call the State Public Health Veterinarian Robert Johnson, DVM, at 802-863-7240, as you normally would with suspect rabies in an animal.
- Brain tissue samples collected for rabies testing can be used for arboviral testing as well. (Note: tissue samples should be refrigerated and kept cold during transport).

\(^1\) Tengelsen, LA et al., “Response to and efficacy of vaccination against eastern equine encephalomyelitis virus in emus” JAVMA Vol. 218, No. 9, 2001
• Brain tissue samples should be sent to the Vermont Health Department Laboratory with the rabies sample submission form.
• If the sample is negative for rabies, the brain tissue sample will be sent to Wadsworth Lab or the NH Department of Health Laboratory for testing for EEEV and WNV.

Animals that are alive:
• **Before submitting samples for arbovirus testing, please call the State Veterinarian’s office at 802-828-2421 to confirm that the case meets the testing criteria.** If the criteria for testing are met, a serum or CSF sample can be sent to the Vermont Department of Health Laboratory. Please use the lab submission form available at [http://healthvermont.gov/enviro/ph_lab/documents/Laboratory_Clinical_Test_Request_Form.pdf](http://healthvermont.gov/enviro/ph_lab/documents/Laboratory_Clinical_Test_Request_Form.pdf)
  
  o Check the box next to “Other” in the Serology Tests (Misc) section and write WNV/EEE on the line next to it.
  o Also write what kind of animal it is somewhere on the form (there is no designated place for this)

• Serum samples
  o Submit ≥ 3 ml of serum. IgM testing for both EEEV and WNV will be done on this sample.
  o Serum should be collected within the first 14 days of illness.
  o Samples positive on the initial screening will be confirmed by PRNT.
  o Convalescent samples may be necessary and should be collected about 2 weeks later.
  o Serology can be difficult to interpret in vaccinated animals.

• CSF
  o Submit ≥ 1 ml of CSF. IgM testing for both viruses will be done on this sample.

Please remember that both WNV and EEE in animals are reportable diseases in Vermont. If you have any questions, please call the State Veterinarian’s office at 802-828-2421 or the Health Department’s Infectious Disease Epidemiology program at 802-863-7240.

For more information on arboviruses and mosquitoes, please go to our websites:
[http://healthvermont.gov/prevent/arbovirus/index.aspx](http://healthvermont.gov/prevent/arbovirus/index.aspx) - or -

Thank you for your help.

Kristin Haas, DVM                        Erica Berl, DVM, MPH
State Veterinarian                        Assistant State Public Health Veterinarian
Vermont Agency of Agriculture             Vermont Department of Health

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1 IgM is species specific and can only be done on equids. For other species, a plaque-reduction neutralization titer will be done. This test is very specific, but it is a total antibody test that doesn’t distinguish IgM from IgG. Therefore, it cannot distinguish a vaccination titer from a disease response or acute disease from a past infection. It may be necessary to collect a convalescent sample to confirm the diagnosis.