Eastern Equine Encephalitis Virus in Florida
Clinical Signs, Diagnosis, Prevention

Maureen T. Long, DVM, PhD
ACVIM-Large Animal
Department of Comparative, Diagnostic, and Population Medicine
College of Veterinary Medicine
June 28, 2018
Sources of Data Sans Human Testing

Encephalitis Suspect/Other Mammals etc

- Mosquito Testing
- Sentinel Chicken Testing

Veterinarian: Testing/Report

Investigation: Confirm Location

Result:
- State Veterinarian (DACS)
- Vector ID

DOH: Medical Alerts

Mosquito Control
Animal Disease in Zoonotics

- Extremely important for surveillance and risk of disease in humans
- Probably contribute more regarding epidemiology
- Clinical signs, dx testing, immune response often similar
- More frequent ability to examine pathology
Alphavirus (EEEV) Surveillance

Veterinary EEEV Disease in Florida, June 2017-2018

Sentinel Seroconversions to EEEV in Florida, 2017-2018
Eastern Equine Encephalitis virus

- Disease of humans and horses
  - One of the most pathogenic neurological viruses on planet
- Mortality lower in humans
  - Horse >90%
  - Spontaneous mortality is probably equal to this
Trend of Nonhuman vs Human Cases

- Nonhuman:human reporting decreasing
- Vaccination
- Select Agent Status
  - Governmentally control of who works on disease
    - This includes diagnostics
  - Few veterinary diagnostic laboratories perform EEEV testing
During active years (2003-2016; Lindsey et al):

- Thirty-one states reported animal species
- 88% of reported cases in nonhuman species
- Animals
  - 97% equids (3,016)
  - 12% camelids
  - 9% canids
  - 6% cervids
  - Others: rhatites*, exotic birds, sheep, goats
Florida Analyses (Long, Gibbs, MacKay)
Case Example

- ~4 yrs, Ranch TW
- Dubious Vaccine Hx
- Bloodwork: hyperammonemnia
  - Theiler’s?
- Eventually became comatose, recumbent
- Performed rabies postmortem
- Rabies (-) EEEV (+++) WNV (-)
<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>% EEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>69-90</td>
</tr>
<tr>
<td>Ataxia</td>
<td>63-90</td>
</tr>
<tr>
<td>Inc. R. Temp</td>
<td>50</td>
</tr>
<tr>
<td>Recumbence</td>
<td>47-90</td>
</tr>
<tr>
<td>Weakness</td>
<td>45</td>
</tr>
<tr>
<td>Fasciculations</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>% EEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aimlessness</td>
<td>28-75</td>
</tr>
<tr>
<td>H. Pressing</td>
<td>17</td>
</tr>
<tr>
<td>Hyperaesthesia</td>
<td>12</td>
</tr>
<tr>
<td>Blindness</td>
<td>8-7</td>
</tr>
<tr>
<td>Seizures</td>
<td>8-20</td>
</tr>
<tr>
<td>Coma</td>
<td>8-90</td>
</tr>
</tbody>
</table>
Age Distribution Similar to Humans

![Graph showing age distribution of horses.](image)

![Graph showing time since vaccination.](image)
Diagnosis

• Complete under reporting!
  • Clinical Signs
  • Most by a single serum test
  • MAC ELISA
    • Mouse-brain antigen
    • Very limited distribution!
    • Need for development of non-SA antigen
  • 4-fold difference on consecutive NT
    • Rarely a horse survives for a second sample
  • 4-fold difference between EEE and WEE on single sample (see Snahu et al, Pederson et al)
• Post-mortem
  • Many horses never reach post-mortem
Cerebrospinal Fluid

- Very specific for EEEV
  - "neutrophilic" response

- When horses are euthanized in the field
  - Can take CSF and serum
  - Will suffice for diagnosis also
Post-mortem Testing

• Must use personal protection equipment!

• Choices in Field
  • Disarticulation of head and ship to pathology/diagnostic laboratory
  • Euthanize, ship whole carcass
Prevention

**Vaccination**

- **Horses:**
  - All foals received three boosters 6, 7, and 9 mos.
  - Mares received a booster one month before foaling.
  - Horses aged 1-5 years should receive 3 injections per year.
  - Older than 5 years, twice per year.

- **Emus and other ratites**
  - Require three injections per year.
  - Brooding females, keep vaccinated for maternal antibody.
  - Young and unvaccinated require three initial injections 3-4 weeks apart starting at 5-6 weeks of age.

- **Dogs**
  - Usually puppies have been reported
  - Limited number of dogs had no response to vaccines
Prevention

- Site cleanup
  - Standing water
  - Removal of junk
  - Removal of old tires
  - Application of “dunks”
  - Treatment of ponds-stagnant, removal of weeds

- Mosquito Control
  - Larvicide treatment
  - Adulticides
  - I use premise sprays in barns and buildings
West Nile virus
Epizootic Period: Establishment

West Nile Virus in 2001 - Equine

733 equine cases detected in 19 states. 66% of cases occurred in Florida
Epizootic Period: Explosive Outbreak

1,406 equine cases detected Most of the cases occurred in the west
2,539 human WNV cases

2002
Endemic Period

Incidence and case counts for equids lower than human case count. Dead bird counts sporadically reported. Limited mosquito testing.

Since 2012 in re-emergent period over 13,500 cases of WNV: 5,674 WN fever/encephalitis cases in 2012 with over 2,000 annually
Other Mammals

- Ewes: fever, abortion, rarely encephalitis
- Pigs: asymptomatic
- Dogs: asymptomatic
- Camelids: Alpaca’s have relatively high virus in the brain!
- Rabbits now used for experimental infection
Clinical Signs - Horses

- 61% Fasciculation and Tremors
  - Head
  - Neck
  - Trunk
- >60% Change in Behavior
- Varies from hyperexcitability to somnolence
Clinical Signs - Horses

- Ataxia and Weakness
Paralysis

• Intermittent Weakness/ Paralysis
• Flaccid Paralysis/Recumbency
Mid- and Hindbrain Involvement

Facial paralysis 39%
Tongue paralysis 20%
• CBC, Chemistry
  • No specific pattern
  • Horses early in disease show dehydration

• Spinal Tap
  • May be very helpful
  • Can indicate an encephalitis
  • Over 90% are abnormal
## WNV CSF Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LS Median (Range)</th>
<th>AO Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>103 (72) 52-316</td>
<td>57 (72) 36-104</td>
</tr>
<tr>
<td>RBC</td>
<td>123 0-5400</td>
<td>2 0-376</td>
</tr>
<tr>
<td>WBC</td>
<td>14 (6) 0-310</td>
<td>6.5 (6) 0-882</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>1 0-14</td>
<td>1.5 0-33</td>
</tr>
<tr>
<td>Lymph (%)</td>
<td>63 0-95</td>
<td>40 0-91</td>
</tr>
<tr>
<td>Mono (%)</td>
<td>29 0-81</td>
<td>54 0-96</td>
</tr>
</tbody>
</table>

Wamsley et al, 2002  N=33
Diagnosis

- Very reliant on single IgM Capture
  - Can be confounded by recent vaccination
  - More recent publications demonstrate confounding
  - Experimental data indicates confounding
- In human testing, IgM confounded to SLE in FL (unless travel history)
  - Our study site in Pakistan of 1000 humans shows extensive cross-reaction to JEV, SLE, ZIKV, DENV 1-4
Post-mortem Confirmation More Difficult Than EEEV

Cerebrum +/-
Thalamus +++
Pons +++
Medulla +++
Cerebellum +/-
Cervical Cord +
Lumbar Cord ++

All Tests Are Dependent On Receiving Sections with Lesions:
Any discoloration, multiple tissues

Nested rtPCR > Real-time rtPCR > Immunohistochemistry > Classic rtPCR
• Acute CNS invasion is leads to hyperexcitation:
  • Glutaminergic activation
    • Excitotoxicity
    • Leads to increase CA++ in neuron, death of neuron if bad enough

• Treatments: Glutamate blocking, Glutamate Scavenging
  • Anti-psychotics?—Clonapine vs Acepromazine?
  • Glutamate receptor upregulators?—Minocycline
    • Will these blockers actually kill more neurons?
Discussion

• Are arbovirus diseases under reported in humans as they are in horses.

• How can we leverage animal/mosquito information better to inform practitioners of enhanced activity?

• Are medical practitioners similar to veterinary practitioners in under testing
  - We DVMs have a systemic bias
  - Also “no cure, no need to test”
Thank-you
4 Serologic Methods

- Presented at the AAVLD Meeting 2007
- Pederson DD, Albers SJ, Klein SM, Ostlund EN
  - USDA-APHIS, NVSL, Ames IA
- Looked at animals from 2005 season
- Compared 66 positive and 22 negative samples by IgM EEE
- Compared to HI, PRNT, CF
- All were then tested against WEE and VEE
Results-Positive IgM Capture

- 66/67 horses were positive to PRNT
  - 61/67 were only positive to EEE
  - 4/67 also positive to WEE
  - 4/67 also positive to VEE
- 59/67 horses were positive to HI
  - 48/67 horses were only positive to EEE
- 7/67 were positive to CF
Results - IgM Negative

- 17 were positive to the PRNT
  - 4 reacted to EEE only
  - 9 reacted to EEE and WEE
  - 3 reacted to VEE - all were from South America

- 16 were positive on the HI
- 8 were positive to the CF
IgM is highly useful on single serum sample
  - Especially for rapid screening
  - In EDART-if IgM positive to EEE, will be low positive to VEE but not WEE
Those samples with a high suspicion and IgM, PRNT would be next useful
  - MUST have prior vaccine history
Do our testing guidelines agree with this?
### Table 4. Equine encephalomyelitis antibody titers in horses that yielded eastern equine encephalomyelitis (EEE) virus.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>ELISA titer to EEE</th>
<th>Hemagglutination inhibition titers</th>
<th>Virus neutralization titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>EEE</td>
</tr>
<tr>
<td>17</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>160</td>
</tr>
<tr>
<td>18</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>40</td>
</tr>
<tr>
<td>19</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>40</td>
</tr>
<tr>
<td>20</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>640</td>
</tr>
<tr>
<td>21</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>160</td>
</tr>
<tr>
<td>22</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>160</td>
</tr>
<tr>
<td>23</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>40</td>
</tr>
<tr>
<td>24</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>&lt;10</td>
</tr>
<tr>
<td>25</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>10</td>
</tr>
<tr>
<td>26</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>40</td>
</tr>
<tr>
<td>27</td>
<td>&gt;1,000</td>
<td>&lt;100</td>
<td>&gt;1,280</td>
</tr>
<tr>
<td>28</td>
<td>&gt;1,000</td>
<td>1,000</td>
<td>40</td>
</tr>
<tr>
<td>29</td>
<td>&gt;1,000</td>
<td>1,000</td>
<td>40</td>
</tr>
<tr>
<td>30</td>
<td>&gt;1,000</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>31</td>
<td>&gt;1,000</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

* Western equine encephalomyelitis.
† Venezuelan equine encephalomyelitis.
‡ ND = not done.
# Cerebrospinal Fluid

<table>
<thead>
<tr>
<th>Protein</th>
<th>EEE</th>
<th>WEE</th>
<th>WNV</th>
<th>Rabies</th>
<th>EPM</th>
<th>EHV</th>
<th>Parasite</th>
<th>Bot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↑</td>
<td>↑</td>
<td>N to ↑↑</td>
<td>N to ↑</td>
<td>Moderate &gt;80 mg/dl</td>
<td>↑↑↑↑</td>
<td>↑↑</td>
<td>N</td>
</tr>
<tr>
<td>Cells</td>
<td>↑↑</td>
<td>N or ↑</td>
<td>N to ↑</td>
<td>N to ↑</td>
<td>N to ↑</td>
<td>N</td>
<td>↑↑</td>
<td>N</td>
</tr>
<tr>
<td>Color</td>
<td>------</td>
<td>------</td>
<td>(Xantho)</td>
<td>(Xantho)</td>
<td>(Xantho)</td>
<td>Xantho</td>
<td>----</td>
<td>N</td>
</tr>
</tbody>
</table>

P=PMN  LM=Lymphs, Monos
Eastern Equine: Hypersegmented Neutrophils
Confirmed EEE Breeds

N=259
<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>% EEE</th>
<th>% WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aimlessness</td>
<td>28</td>
<td>---</td>
</tr>
<tr>
<td>H. Pressing</td>
<td>17</td>
<td>1-2</td>
</tr>
<tr>
<td>Hyperaesthesia</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>Blindness</td>
<td>8</td>
<td>1-2</td>
</tr>
<tr>
<td>Seizures</td>
<td>8</td>
<td>1-2</td>
</tr>
<tr>
<td>Coma</td>
<td>8</td>
<td>---</td>
</tr>
</tbody>
</table>