La Crosse Encephalitis in Eastern Tennessee

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Clinical Presentation and Epidemiology

- Fever, headache, nausea, vomiting, fatigue, lethargy.
- Meningitis, encephalitis
- Neurological sequelae: recurrent seizures, hemiparesis, cognitive and neurobehavioral abnormalities
- Case fatality less than 1%
- Supportive therapy (including seizure control)

- Most cases in children under 16
- Average 80-100 cases reported per year
- Most cases July - September
- Boys mostly affected
Distribution of La Crosse Encephalitis Cases, by Month of Onset, Tennessee, 1998-2010

Number of Cases

Month of Onset

January
February
March
April
May
June
July
August
September
October
November
December
California Serogroup Virus Neuroinvasive Disease Cases, 1964-2010
California Serogroup Virus Neuroinvasive Disease
Average Annual Incidence by County, 1996-2010
Unsmoothed risk at county level for children 15 and under, 2003-2007


Distribution of unsmoothed risk of La Crosse virus infections at the county level for eastern Tennessee of population 15 years and younger, 1997-2006

Emergence in Tennessee

- From 1964-1996, only 9 cases in TN
- 1997 cluster of 10 cases in eastern TN
- Cases increased about the same time in WV and NC
- Over 160 cases reported in TN
Vectors

- **Ochlerotatus triseriatus**
  - Treehole mosquito
  - Primary vector

- **Aedes albopictus**
  - Asian tiger mosquito
  - New introduction

- **Ochlerotatus japonicus**
  - Asian bush mosquito
  - Newer introduction
Hypothesis

- These mosquito species may differ in their relative contribution to the maintenance and transmission of LACV to humans
  - Differences in abundance at case sites
  - Differences in infection rates at case sites
  - Differences in blood meal composition at case sites
- Some traps are better than others for capturing specific species
Methods
Case reports were obtained for all LACV cases from 2004-2009

Cases were mapped and clusters identified

Calls were made to households in the order of most recent and in our preferred counties (Knox and Claiborne)

Six households were enrolled representing five cases
Egg Collections and Rearing

- Five standard oviposition cups were set at each site.
- The eggs are removed each week and returned to the lab.
- Eggs are reared to adults in a temperature controlled environmental chamber.
Larval Collections

- All standing water on the field sites is examined every week for larvae/pupae
- If present, a sample is collected and returned to the lab for rearing
Adult Collections

- Adults are collected in 2 ways: the BG Sentinel Trap and by aspirating
- The BG trap is set every week and run for approximately 24 hours
- Each site is aspirated, using either the Prokopack or the CDC Backpack Aspirator, for 20-40 minutes 1 to 4 days a week
- Adults are transported back to the lab on dry ice
Identification and Storage

- Adults are identified, sorted and numbered using a compound microscope and a chill table.
- They are separated into pools of ≤ 23 mosquitoes by site, date, species and sex.
- Stored in a -80°C chamber.
Mosquito pools are homogenized in cell culture media
RNA is extracted using QIAamp Mini RNA Extraction Kit or Biogents Robot
RT-PCR is run using the protocol from Kuno et al. 1996
The primers screen for 24 different viruses in the Bunyamwera/California groups, including JC
Some samples have also been tested using cell culture
## Species per trap

<table>
<thead>
<tr>
<th>Trap</th>
<th>Species</th>
<th># mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prokopack</td>
<td>Oc. triseriatus</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Ae. albopictus</td>
<td>276</td>
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<tr>
<td></td>
<td>Oc. japonicus</td>
<td>0</td>
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<tr>
<td>BG trap</td>
<td>Oc. triseriatus</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td>Ae. albopictus</td>
<td>2374</td>
</tr>
<tr>
<td></td>
<td>Oc. japonicus</td>
<td>94</td>
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<tr>
<td>Ovitrap</td>
<td>Oc. triseriatus</td>
<td>12400</td>
</tr>
<tr>
<td></td>
<td>Ae. albopictus</td>
<td>3485</td>
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<tr>
<td></td>
<td>Oc. japonicus</td>
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<tr>
<td>Larval collections</td>
<td>Oc. triseriatus</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Ae. albopictus</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>Oc. japonicus</td>
<td>208</td>
</tr>
</tbody>
</table>
Mosquitoes Collected

**BG Sentinel, 2010**

- **Aedes albopictus**
- **Ochlerotatus triseriatus**

**Ovicups, 2010**

- **Aedes albopictus**
- **Ochlerotatus triseriatus**
Infected *Ochlerotatus triseriatus* (ovicups)

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>Gender</th>
<th># Pools</th>
</tr>
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<tbody>
<tr>
<td>6/9/2010</td>
<td>1</td>
<td>M</td>
<td>6</td>
</tr>
<tr>
<td>6/9/2010</td>
<td>1</td>
<td>F</td>
<td>4</td>
</tr>
<tr>
<td>6/22/10</td>
<td>5</td>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>6/22/10</td>
<td>5</td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>7/12/10</td>
<td>6</td>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>7/19/10</td>
<td>1</td>
<td>M</td>
<td>1</td>
</tr>
<tr>
<td>7/19/10</td>
<td>2</td>
<td>F</td>
<td>3</td>
</tr>
<tr>
<td>7/19/10</td>
<td>3</td>
<td>M</td>
<td>3</td>
</tr>
<tr>
<td>7/19/10</td>
<td>5</td>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>8/9/10</td>
<td>5</td>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>8/9/10</td>
<td>6</td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>8/19/10</td>
<td>3</td>
<td>F</td>
<td>8</td>
</tr>
<tr>
<td>8/19/10</td>
<td>5</td>
<td>M</td>
<td>2</td>
</tr>
</tbody>
</table>
### Other LACV Infected Mosquitoes

<table>
<thead>
<tr>
<th>Species</th>
<th>Gender</th>
<th>Site</th>
<th>#/pool</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ochlerotatus japonicus</em></td>
<td>F</td>
<td>6</td>
<td>20</td>
<td>5/19/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>F</td>
<td>1</td>
<td>3</td>
<td>5/24/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>M</td>
<td>3</td>
<td>13</td>
<td>6/1/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>F</td>
<td>5</td>
<td>18</td>
<td>7/7/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>M</td>
<td>6</td>
<td>9</td>
<td>7/12/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>M</td>
<td>1</td>
<td>5</td>
<td>7/19/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>M</td>
<td>3</td>
<td>8</td>
<td>7/19/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>F</td>
<td>1</td>
<td>7</td>
<td>7/27/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>F</td>
<td>5</td>
<td>18</td>
<td>8/9/2010</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>M</td>
<td>5</td>
<td>17</td>
<td>8/9/2010</td>
</tr>
</tbody>
</table>
Bloodmeal

- What are they eating?
  - Samples: 15 *Aedes albopictus*, 2 *Ochlerotatus triseriatus*, 1 *Ochlerotatus japonicus*
  - Tested for mammalian and avian bloodmeals
Hypothesis

- These mosquito species may differ in their relative contribution to the maintenance and transmission of LACV to humans
  - Differences in abundance at case sites
    - Trap dependent, *Oc. triseriatus* > *Ae. albopictus* > *Oc. japonicus*
  - Differences in infection rates at case sites
    - 11 LACV ID events for *Oc. triseriatus* and 8 for *Ae. albopictus*
    - 1 LACV ID event for *Oc. japonicus*
  - Differences in blood meal composition at case sites
    - Testing pending

- Some traps are better than others for capturing specific species
  - *Oc. triseriatus* = Ovicups
  - *Ae. albopictus* = Ovicups/BG
  - *Oc. japonicus* = Ovicups
Conclusions

- 1st ID of LACV in *Oc. triseriatus* in TN
- 1st ID of LACV in *Oc. japonicus* in U.S.
- 2nd ID of LACV in *Ae. albopictus* in TN (3rd in U.S.)
- For surveillance
  - BG may be useful for *Ae. albopictus*
  - Ovicups for *Oc. triseriatus* and *Oc. japonicus*
- Prokopack good for bloodmeal collections in *Ae. albopictus*
- Larval collections representative of populations
- PCR more sensitive than cell culture assays
Acknowledgements

- TDH
  - Katie Westby
  - Melissa Heim
  - Erin Westby
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  - Charissa Fritzen
  - Junjun Huang

- University of Tennessee
  - Dave Paulson
  - Dr. Carl Jones

- The families in east TN
Questions?