La Crosse in NC: Evaluating the CDC AGO

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La Crosse Virus

- Isolated in 1960’s in La Crosse, Wisconsin
  - Bunyavirus (California serogroup virus)
- Only acquired through the bite of a mosquito
  - Eastern-tree hole mosquito (principle vector)
- LACv is the most common arboviral cause of pediatric encephalitis in the US
Although LACE was historically found throughout the Midwest, the burden has shifted to the Appalachian region: 81% reported from Ohio, West Virginia, North Carolina, and Tennessee (2003-2012). Within NC, the western counties have the highest incidence rate of LACE. Most cases occur in Buncombe, Haywood, Henderson, Jackson, Macon, Swain, and Transylvania counties.
Arboviral Disease (Mosquito Transmitted) NC: 2006-2015

LACE: La Crosse Encephalitis
WN: West Nile (Neuroinvasive disease)
EEE: Eastern Equine Encephalitis

North Carolina cumulative human disease cases reported to CDC ArboNET

2015: Preliminary Data
LACv Infection

Largely Unrecognized

- Most infections: Asymptomatic
- Each recognized case: ~200 infections
- “Tip of the Iceberg”

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macon Co.</td>
<td>36</td>
<td>8.3</td>
</tr>
<tr>
<td>Swain Co.</td>
<td>175</td>
<td>8.0</td>
</tr>
<tr>
<td>Jackson Co.</td>
<td>225</td>
<td>4.9</td>
</tr>
<tr>
<td>Haywood Co.</td>
<td>162</td>
<td>2.5</td>
</tr>
</tbody>
</table>

La Crosse Virus Cycle

Small Mammals
- *Tamias striatus*
- *Sciurus carolinensis*

Mosquito Vector
- *(Ae. triseriatus)*

Small Mammals
- *Tamias striatus*
- *Sciurus carolinensis*

Mosquito Vector
- *(Ae. triseriatus)*

Human Host
- (“Dead End”)

Transovarial Transmission

Infected Progeny
- (Male and Female)

(Virus dissemination/Transovarial transmission)

Female Mosquito
- *(Ae. triseriatus)*

Male Mosquito
- *(Ae. triseriatus)*

Female Mosquito
- *(Ae. triseriatus)*

Venereal Transmission
- (male to uninfected female)

Adapted from Beaty and Marquardt (1996)
Invasive Vectors

Aedes albopictus: “Asian Tiger Mosquito”
- Can transmit La Crosse virus
- Readily feeds on Humans
- Aggressive, Daytime Feeder

Aedes japonicus: “Asian Bush Mosquito”
- Can transmit La Crosse virus
- Feeds on Humans
- Less Aggressive, Daytime/Evening Feeder

East TN: LACv IRs for Ae. japonicus (0.63) were lower than Ae. triseriatus (2.72) and Ae. albopictus (3.01) (Westby et al., 2015)
Trapping methods

- **CDC light traps (baited)**
  - Physiologically biased
  - Battery-powered

- **BG-Sentinel**
  - Physiologically biased
  - Battery-powered

- **Gravid Traps**
  - Physiologically biased
  - Battery-powered

- **Nasci aspirator**
  - Limitations: energy and time intensive strategy

- **Some utility for surveillance - ineffective for control**

![Image of a large-bore “Nasci” Aspirator](image-url)
Autocidal Gravid Ovitrap

- Designed by the CDC for DENv Control
  - Vector: Ae. aegypti
  - Hay infusion: Microbial Cues
- Lure gravid females
  - Previously blood fed
  - Greater virus risk (Except TOT)
- Affordable, passive, low maintenance
Aim 1

1. Determine the efficacy of AGO for LACv surveillance
   
   Question: Will the AGO trap Ae. *triseriatus*, Ae. *albopictus*, and Ae. *japonicus*?

2. Compare attractiveness of a White Oak (WO) infusion versus the standard hay infusion

   Question: Does infusion type matter?
Methods

- **Infusion Preparation:**
  - WO (84 g WO leaves/10 L) and hay (30 g hay/10 L) sachets added to water-filled AGOs
- AGOs (n=36) deployed in a balanced, randomized block design at 6 peridomestic sites for 5 weeks (630 trap days per infusion type)
- 6 traps (3 replicates per infusion type) per block
Assign subjects to groups
Each column shows the assignments for a block. For example, the third column for the second row (not counting headings) shows the group assignment of the second subject of the third block.

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>Block 4</th>
<th>Block 5</th>
<th>Block 6</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
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<tr>
<td>2</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
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<td>5</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

How it works: The random number generator is seeded with the time of day, so it works differently each time you use it. Each subject is first assigned to a group nonrandomly. Then the assignment of each subject is swapped with the group assignment of a randomly chosen subject. This should suffice, but the entire process is repeated twice to make sure it is really random. Note that you can copy and paste the values from the web page into Excel.

Infusion Type
O = Oak
H = Hay

Distance between traps = 5 meters
Methods

- Mosquitoes collected and processed
  - Enumerated, identified by morphology and sex
  - Physiological status determined (e.g., gravid, parity, etc.)
- Molecular techniques
  - *Ae. triseriatus* vs. *Ae. hendersoni* (Wilson et al., 2014)
  - Unknowns
    - Damaged/unidentifiable
    - PCR assay with universal mosquito primers
    - ITS2 size
Infusion by Species

- CDC-AGO highly specific for the three targeted LACv vectors (98.7%)
  - *Ae. triseriatus* (52.9%)
  - *Ae. japonicus* (38.1%)
  - *Ae. albopictus* (7.7%)

- 88% gravid

\[
\chi^2 (2) = 1.627, p = .443
\]
Species-Specific Mosquito Collections (Week – Mean #s)

First Week (Priming Week) was Productive (~25% overall)
Oak vs Hay

- **Aedes triseriatus**: Hay collected more than Oak
  - $\chi^2 (1) = 6.25, p = .012$
- **Aedes japonicus**: No difference between Hay & Oak
- **Aedes albopictus**: Too few collected

- The “infusion” was specified as a fixed effect and the “block” and “trap location” were specified as random effects. The Poisson GLMM expressed:
  \[
  \log\{E(Y_{ij} | b_i, l_{ij})\} = \alpha + x_{ij}\beta + b_i + l_{ij},
  \]
- Where $b_i \sim N (0, \sigma^2_b)$ and $l_{ij} \sim N (0, \sigma^2_l)$ represented the random effects of blocks and trap locations, respectively; $x$ indicated which infusion was used, and $\alpha$ was the intercept.
Results

- Mean yield of LACv vectors 0.84 mosquitoes per trap per week
- Slightly lower than observed in Dengue control (Barrera et al, 2014)
  - DEN AGO Study: Mean yield 1.2 mosquitoes per trap per week
- Study Differences
  - Vectors
  - Distribution
  - Density of traps
Additional Work

• **Work In Progress**

• **Microbial Community Profiles**
  – BioLog Plates
  – Variability in microbial community
  – Temporal and Spatial Differences

• **Small Field Trial (Aug. 2015)**
  – 4 traps/house and paired “control”
  – Resting mosquitoes (Nasci)
  – ? Outcomes:
    • Population structure
    • Abundance
Team/Acknowledgements

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WCU School of Health Sciences
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Thank you!

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