Culex pipiens complex (Diptera: Culicidae) host feeding patterns in Sacramento and Yolo Counties

Matthew Montgomery
LTJG MSC USN
2010
Introduction

- Significance
- Background
  - West Nile Virus
  - *Cx. pipiens* complex
- Research Goals/Hypothesis
- Collection and Host Identification Methods
- Results
- Conclusion
Introduction

• Determine the hosts of local Cx. pipiens complex
  • Better knowledge of host feeding patterns to establish its potential as a disease vector for humans
  • West Nile Virus prevention

• Sacramento Yolo Mosquito and Vector Control District (SYMVCD) funded study
Significance

• Insights on a species of global importance
• Implications for vector control and public health in California
  – *Cx. pipiens* is an established vector of WNV
• Vector-host interactions’ role in virus transmission
  – Local host-feeding patterns are unknown
  • Its role as a vector of WNV to humans cannot be evaluated without this information
West Nile Virus

- Flavivirus
- Spread through biological transmission
  - Bird-mosquito enzootic transmission cycle
  - Humans and other mammals in epidemic cycle
    - Humans and most mammals are dead-end hosts
West Nile in Sac-Yolo

- Isolated in New York, 1999
- First detected locally in 2004
  - 22 human cases in 2007
  - 15 human cases in 2008
  - 2 human cases in 2009
- Detected in birds, humans, horses, and mosquitoes
  - Majority of positive birds are Corvids
California 2008 WNV Incidences
Culex pipiens

• The house mosquito
• Breeds in foul, nutrient rich waters
• World-wide distribution
  – Found on every continent but Antarctica
  – In temperate, tropical, and subarctic habitats
Cx. pipiens and West Nile Virus

• Established WNV vector (Reisen et al. 2005)
  – Initiates transmission 6-7 days after taking infected bloodmeal (Goddard et al. 2003A)
  – Low rates of vertical transmission (Goddard et al. 2003B)
Cx. pipiens Host-Feeding Patterns

• Primary hosts in CA thought to be avian
  – Not well established
  – Nearest study to Sac-Yolo was in Kern County (Reeves 1990)

• Readily feeds on humans in other parts of the world
  – Major human SLE and WNV vector on East coast (Monath 1980; Turell et al. 2005)
  – Anthropophilly well documented in Soviet Union (Vinogradove 2000)
**Cx. pipiens Complex**

- Species complex with diverse behaviors and host feeding preferences
- *Cx. pipiens* complex in CA:
  - *Cx. p. pipiens* in north
  - *Cx. p. quinquefasciatus* in south
  - Hybridization throughout the Central Valley (Reeves 1990)
- Males once thought to be morphologically distinguishable
  - Recent genetic work has shown this not to be the case (McCabee et al. 2008)
Research Hypothesis

The *Culex pipiens* complex in Sacramento and Yolo counties feeds on a wide-range of vertebrate host species allowing it to function as both an amplifying and bridge vector of West Nile Virus.
Methods

Study Area

• Mosquito collections strictly limited to Sacramento and Yolo counties
  – Combined area of 2,018 sq mi
  – Human population ~1.4 million
  – Diverse habitats: wetlands, rangelands, farmlands, suburban and urban centers
  – WNV present since 2004
Collection

• Blood-engorged *Cx. pipiens* complex collected from field
• Attempt to obtain majority of samples from areas with abundant human hosts
  – Focus on urban and suburban areas
  – Rural and agricultural sites sampled as well
  – Red resting boxes also used
Collection

- Collected mainly from natural resting sites
  - Under bridges, awnings, public restrooms etc.
Red Resting Boxes
Collection

- Mosquitoes collected by backpack aspirator and vacuum aspirator
- Date and location of capture recorded
- Identified to Cx. *pipiens* complex level in lab
- Stored in individual marked vials at -80°C
Collection

• 452 Specimens collected
  – 244 in June-August of 2007
  – 208 in May-September of 2008
    • July had highest mosquito abundance for both years
• Engorged specimens from 31 different sites
  – 22 Urban/Suburban (298 specimens)
  – 9 Rural (154 specimens)
• Collections biased towards areas with human hosts present
Bloodmeal Host Identification

• Numerous well-developed methods available
  – ELISA is simple and most common, but lacks specificity
  – Limited antibody availability

• Recently assays based on host genome sequences have been developed
  – Excellent specificity for a wide range of hosts
CO1 Sequencing Method

• Utilizes the Cytochrome Oxidase 1 gene of mitochondrial DNA
  – CO1 codes for a protein in the mitochondrial Electron Transport Chain
  – Little intraspecies variation
  – Adequate interspecies variation among vertebrates, but with enough conserved regions for universal primer design
Sequencing Method

• Engorged abdomen cut from thorax and ground in phosphate buffered saline
  – Note: head and thoraces stored at -80°C, so that future investigators can determine which member of the complex they are
• DNA extracted with “Qiagen DNeasy ® Blood and Tissue Kit”
• PCR utilizing vertebrate CO1 primers described by Cooper et al. (2007)
• Forward and reverse consensus sequences on Sequencher® program
• Sequences run in the Barcode of Life Data Systems (BOLD) Identification Engine (http://www.barcodinglife.com)
  – Returns a taxonomic identification to the species level
<table>
<thead>
<tr>
<th>Bird Species</th>
<th>✓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Scrub-Jay</td>
<td>✓</td>
</tr>
<tr>
<td>American Crow</td>
<td>✓</td>
</tr>
<tr>
<td>Yellow-billed Magpie</td>
<td>✓</td>
</tr>
<tr>
<td>Song Sparrow</td>
<td>✓</td>
</tr>
<tr>
<td>House Sparrow</td>
<td>✓</td>
</tr>
<tr>
<td>House Finch</td>
<td>✓</td>
</tr>
<tr>
<td>European Starling</td>
<td>✓</td>
</tr>
<tr>
<td>Cliff Swallow</td>
<td>✓</td>
</tr>
<tr>
<td>Red-winged Blackbird</td>
<td>✓</td>
</tr>
<tr>
<td>Mourning Dove</td>
<td>✓</td>
</tr>
<tr>
<td>Rock Pigeon</td>
<td>✓</td>
</tr>
<tr>
<td>Chicken</td>
<td>✓</td>
</tr>
<tr>
<td>Snowy Egret</td>
<td>✓</td>
</tr>
</tbody>
</table>
Results

- 330 bloodmeals identified to host species
  - 25 host species (24 avian, 1 mammalian)
  - 99.1% of all bloodmeals avian
  - 3 feedings on cows
  - No human feeding
## Major Avian Hosts

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Robin</td>
<td>57</td>
<td>17.3</td>
</tr>
<tr>
<td>House Finch</td>
<td>51</td>
<td>15.5</td>
</tr>
<tr>
<td>Barn Swallow</td>
<td>44</td>
<td>13.3</td>
</tr>
<tr>
<td>Western Meadowlark</td>
<td>40</td>
<td>12.1</td>
</tr>
<tr>
<td>Mourning Dove</td>
<td>39</td>
<td>11.8</td>
</tr>
<tr>
<td>House Sparrow</td>
<td>25</td>
<td>7.6</td>
</tr>
<tr>
<td>Brewer’s Blackbird</td>
<td>18</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Seasonal Shifts

- Brewer’s Blackbird
  - June of 2007 vs. all other months ($p=0.008$)

- Barn Swallow
  - Major host during early summer

- American Robin
  - Significantly higher % of bloodfeeding in late summer ($p=0.03$)
American Robin

• Most common host (17.3%)
• 21 of 28 sites
• Seasonal shift (Hamer et al. 2009; Kilpatrick et al. 2006a; Kilpatrick et al. 2006b; Savage et al. 2007)
  – % Robins declined over summer
• My results:
  – Robin derived bloodmeals increased significantly over the summer
American Robin

Percentage of *Cx. pipiens* complex bloodmeals from *T. migratorius* Hosts

- 2008 Collections
- 2007 Collections
American Robin

- Influx of post nesting migrants?
- High WNV transmission competence (Komar et al. 2003)
-Potentially important role in local WNV amplification
Major Hosts

- **House Finch (15.5%)**
  - Consistent across seasons (all months >10%)
  - Important rural and non-rural host
  - High WNV transmission competence (Reisen et al. 2005)

- **House Sparrow (7.6%)**
  - Competent WNV reservoir (Reisen et al. 2005)
  - Mainly associated with Barn Swallow
Major Hosts

• Mourning Dove (11.8%)
  – Only major non-Passeriform host
  – Mainly non-rural host
  – Moderate WNV transmission (Reisen et al. 2005)
Major Hosts

- Western Meadowlark (12.1%)
  - Major non-rural host
    - Normally associated with open grasslands
    - Found in non-rural sites associated with nearby meadows
  - Transmission competence not established
  - Dead birds have tested positive
Major Hosts

- Barn Swallow (13.3%)
  - Major rural host for short time periods
  - Transmission competence not established
Corvids

- Accounted for less than 3% of bloodmeals
  - Western Scrub Jay (6)
  - American Crow (2)
- Most common WNV positive dead birds in district
Conclusion

• Host feeding patterns as a means of determining public health risk
• Based on bloodmeals at the times and places I collected:
  – *Cx. pipiens* is likely not an epidemic vector of WNV to humans and other mammals
  – Mainly epizootic transmission role
  – Supports idea of certain preferred avian hosts
Acknowledgements

• Dr. Tom Scott, Dr. William Reisen, and Dr. Anton Cornel
• Sacramento Yolo Mosquito and Vector Control District, especially Dr. Paula Macedo and Marcia Reed
• Special thanks to Tara Thieman and Jacklyn Wong for their help and encouragement