Mosquito-Borne Disease Surveillance in Iowa: Collaborative Benefits Beyond Human Case Surveillance

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West Nile virus (WNV)

- *Flavivirus*
  - Single stranded RNA virus

- Leading cause of mosquito-borne disease in the continental United States
  - In 2018, 48 states and the District of Columbia reported WNV human infections
  - 2,544 human WNV disease cases (CDC 2018 provisional data)

- Infects humans, birds, mosquitoes, horses, and other mammals
Brief History of “Firsts” for WNV

- Identified in 1937 in the West Nile district of Uganda
- Recognized in 1951 epidemic of WNV in Israel
- Recognized in US in New York City in 1999
- Identified in Iowa in a dead crow in the eastern part of the state in September 2001
- SHL received funding from CDC for enhanced surveillance testing in 2000
- Human case in Iowa identified in 2002
West Nile virus

- Most commonly spread to people by the bite of an infected mosquito
  - Mosquitoes become infected when they feed on infected birds

- Uncommon means of transmission:
  - Exposure in a laboratory setting
  - Blood transfusion and organ donation
  - Mother to baby, during pregnancy, delivery, or breast feeding
West Nile virus

- Incubation period ranges from 2 to 14 days, but is typically 2 to 6 days
- 80% individuals infected are asymptomatic
- 20% individuals infected are symptomatic

- Non-neuroinvasive
  - Fever with other symptoms such as headache, body aches, joint pains, vomiting, diarrhea, or rash

- Neuroinvasive (less than 1% of infected persons)
  - High fever, headache, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness and paralysis
  - Develop severe illness affecting the central nervous system such as encephalitis, meningitis, or acute flaccid paralysis.
West Nile virus in Iowa

Current surveillance efforts in Iowa

- **Human infections**
  - Reportable disease
  - Blood banks
  - Local partners

- **Equine infections**
  - The Iowa Department of Agriculture and Land Stewardship

- **Mosquito surveillance**
  - Local partners
  - Iowa State University
  - State Hygienic Laboratory
West Nile virus in Iowa

611 total cases

326% change from 5-year average
West Nile virus in Iowa, 2018

Summary of 2018 WNV cases

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>104</td>
</tr>
<tr>
<td>Incidence rate (per 100,000)</td>
<td>3.4</td>
</tr>
<tr>
<td>Deaths</td>
<td>9</td>
</tr>
</tbody>
</table>
West Nile virus in Iowa, 2018

<table>
<thead>
<tr>
<th></th>
<th>Neuroinvasive</th>
<th>Non-neuroinvasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>59 (57%)</td>
<td>45 (43%)</td>
<td>104</td>
</tr>
</tbody>
</table>

**Gender**

<table>
<thead>
<tr>
<th></th>
<th>Neuroinvasive</th>
<th>Non-neuroinvasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>35 (59%)</td>
<td>28 (62%)</td>
<td>63 (61%)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (41%)</td>
<td>17 (38%)</td>
<td>41 (39%)</td>
</tr>
</tbody>
</table>

**Race**

<table>
<thead>
<tr>
<th></th>
<th>Neuroinvasive</th>
<th>Non-neuroinvasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>58 (98%)</td>
<td>44 (98%)</td>
<td>102 (98%)</td>
</tr>
<tr>
<td>Native Hawaiian or Other Pacific Islander</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

**Ethnicity**

<table>
<thead>
<tr>
<th></th>
<th>Neuroinvasive</th>
<th>Non-neuroinvasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Hispanic</td>
<td>56 (95%)</td>
<td>45 (100%)</td>
<td>101 (97%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (3%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

**Age**

<table>
<thead>
<tr>
<th></th>
<th>Neuroinvasive</th>
<th>Non-neuroinvasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>60.2</td>
<td>43.8</td>
<td>53.1</td>
</tr>
<tr>
<td>Median</td>
<td>63.0</td>
<td>42.0</td>
<td>56.5</td>
</tr>
<tr>
<td>Range</td>
<td>12-90</td>
<td>10-75</td>
<td>10-90</td>
</tr>
</tbody>
</table>
West Nile virus in Iowa, 2018
Contact Information

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Historical Arbovirus Testing at SHL

Arbovirus

- Blood-feeding arthropods (mosquitoes, sand flies, ceratopogonids "no-see-ums", and ticks) that are vector hosts of arthropod-borne viruses
- Sentinel chicken flocks throughout different parts of the state were used to monitor arboviruses since…1966
- Serology lab tests determined if chickens had been exposed and developed antibodies to arboviruses
West Nile Testing at SHL

- 2000 New CDC funding
- Increased trapping and testing of mosquitoes
- Testing sentinel chicken flocks located at 12 sites throughout the state for seroconversions, add WNV. Ended in 2013
- Plan for testing of dead birds identified throughout the state using PCR (assay validation began)
- Serological testing of human sera to detect encephalitis cases
Wild Bird Surveillance

2001 SHL began testing wild birds using Polymerase Chain Reaction (RT-PCR)

Dr. Lanciotti 2000 method

- Test birds without obvious cause of death (corvids-crows and blue jays)
- Isolate WNV RNA from brain tissue
Dead Bird WNV PCR

- Birds were collected in bags with twist ties then sent to SHL
- Lovely smells and piles of maggots
- Cracking skulls - with pliers
- Sweaty PAPR - powered, air-purifying respirator
- 1st Bird (crow) tested positive in Iowa on Sept. 14th 2001 from Scott County
Mosquito Testing by PCR

- Traps were placed at geographically distinct locations around Iowa.
- Mosquito collection started July 9, 2002 and continued through September 26, 2002 (year 1) and every summer since.
- Mosquitoes were pooled, between 1-25 mosquitoes per pool, by species, collection site, and date at ISU and then were sent to UHL for testing.
Mosquito Testing by PCR

- Mosquitoes were originally sonicated, but are now ground with bead beating
- Then nucleic acid (DNA) is extracted (with WNV it is RNA)
- Real-time RT-PCR is performed, or more generically called PCR
  - Viral nucleic acid is primed and doubled each cycle with fluorescent detection
  - Positive mosquitoes usually have a very high viral load, so signal is strong
Mosquito Testing by PCR

- Originally pools of mosquitoes were in pools of 1-50 (in each tube processed)
- Later pools up to 100
- Testing was performed for several years in Ankeny lab and returned to Coralville in 2013
- Optimization- better PCR machines, master mix, pooling, bead beater
Mosquito Pooling

- In 2013 Erik Twait at SHL came up with the idea to pool the pools as a way to save costs.
- WNV viral load is high even in pools.
- SHL verified that pooling pools does not miss positives.
- From 2013-2016 Erik demonstrated an average of 59% cost savings in the reduction of extractions and PCR reactions performed.
Mosquito Pooling

- The individual pools are all bead beaten which produces a slurry.
- Slurry from smaller pools is then re-pooled before nucleic acid extraction.
- PCR is performed on the extracted nucleic acid from pooled pools.
- If pooled pool is positive then all pools are re-extracted individually from their respective slurry and PCR performed.
Mosquito Testing 2018

- The 6 counties surveyed in 2018 were Blackhawk, Monona, Polk, Sac, Story, and Woodbury
- In 2018, 1419 pools were tested, each ranging from 1-50 mosquitoes in each pool
- Provides general surveillance if West Nile is circulating within mosquitoes
- Positivity rate is highest in July-August
Mosquito Testing
Positivity Rate

- Positivity rate doubled from 2015/2016 to 2017/2018

<table>
<thead>
<tr>
<th>Surveillance Year</th>
<th>Total Pools Tested</th>
<th>WNV Positive Pools</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>526</td>
<td>17</td>
<td>3.23%</td>
</tr>
<tr>
<td>2016</td>
<td>1336</td>
<td>46</td>
<td>3.44%</td>
</tr>
<tr>
<td>2017</td>
<td>1453</td>
<td>88</td>
<td>6.06%</td>
</tr>
<tr>
<td>2018</td>
<td>1419</td>
<td>102</td>
<td>7.19%</td>
</tr>
</tbody>
</table>
## Mosquito Testing 2018 Positivity by County

<table>
<thead>
<tr>
<th>County</th>
<th>Total Pools Tested</th>
<th>WNV Positive Pools</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackhawk</td>
<td>57</td>
<td>6</td>
<td>10.53%</td>
</tr>
<tr>
<td>Monona</td>
<td>159</td>
<td>6</td>
<td>3.77%</td>
</tr>
<tr>
<td>Polk</td>
<td>863</td>
<td>78</td>
<td>9.04%</td>
</tr>
<tr>
<td>Sac</td>
<td>59</td>
<td>1</td>
<td>1.69%</td>
</tr>
<tr>
<td>Story</td>
<td>158</td>
<td>5</td>
<td>3.16%</td>
</tr>
<tr>
<td>Woodbury</td>
<td>123</td>
<td>6</td>
<td>4.88%</td>
</tr>
</tbody>
</table>
Pooled Pools 2018

- In 2018 ISU sent 1419 tubes of pooled mosquitoes to SHL.
- All small pools (typically 5-10) are pooled (pooled pools).
- This allowed us to extract and test only 159 pools originally.
- Positive pooled pools were re-tested individually.
- Cuts our costs at least in half (good stewardship of our tax dollars).
State Hygienic Laboratory at the University of Iowa

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Mosquito surveillance in Iowa
Mosquito surveillance in Iowa

The Medical Entomology lab at Iowa State University has analyzed mosquito populations in the state for ~50 years

- **Lyric Bartholomay** (2005-2015)
- **Ryan Smith** (2015-present)
Mosquito surveillance in Iowa

Mosquito trapping occurs from May to October

- **Major questions:**
  - Mosquito population dynamics
    - Numbers
    - Species
  - Monitoring for the introduction of new species
    - *Aedes albopictus*
  - Mosquito-borne disease monitoring
    - West Nile virus (WNV)
Mosquito surveillance in Iowa

Trapping efforts rely heavily on interactions with local county public health personnel

- **In 2018:**
  - WNV surveillance
    - 6 counties
  - Invasive *Aedes* species surveillance
    - 11 counties

>250,000 mosquitoes were collected in 2018!
Mosquito trapping - WNV

In each county, multiple traps are used to collect mosquitoes:

- **New Jersey light trap (NJLT)**
- **CDC light trap**
- **Gravid trap**

Mosquito abundance leads to WNV testing on mosquitoes.
WNV surveillance pipeline

County → Identify mosquitoes
             Compile data

Iowa State University
Mosquito abundance data are publicly shared

https://mosquito.ent.iastate.edu/ - 2018 Data

- These data have three major uses:
  - Inform yearly population trends
  - Define different mosquito ecologies in the state
  - Population trends help partners to enhance mosquito control practices
Mosquito samples for virus testing

Iowa has 55 different mosquito species, yet <10 species have been implicated in WNV transmission

- Using different trapping methods, we focus on 3 major species
  - Culex pipiens
  - Culex restuans
  - Culex tarsalis
WNV surveillance pipeline

1. County
2. Identify mosquitoes
3. Compile data
4. Mosquito pools
5. WNV testing
WNV surveillance outcomes

An increased understanding of WNV transmission in the state

- Iowa 2002-2016

Human WNV cases are strongly associated with regions of western Iowa
WNV surveillance outcomes

An increased understanding of WNV transmission in the state

- Iowa 2002-2016

*Cx. tarsalis* abundance is tightly correlated with human WNV cases

*Cx. pipiens* group
WNV surveillance outcomes

The intensity of WNV in mosquito populations closely match that of human disease cases

- Iowa 2002-2016

Mosquito infection rates enable WNV surveillance in real time to assess human risk
WNV surveillance outcomes

Close monitoring can predict an outbreak before human cases

- Central Iowa 2018

2018 displayed highly increased mosquito infection rates over past years
WNV surveillance outcomes

Monitoring led to increased media outreach to inform the public of potential outbreak

- Central Iowa 2018

Media engagement

Interactions with county public health
- Push for increased mosquito control
WNV surveillance outcomes

Early interventions can reduce potential human WNV cases

- Central Iowa 2018
WNV surveillance summary

WNV monitoring requires close relationships with multiple entities that must work together in a timely fashion

- This directly impacts the public health of Iowans, enabling timely responses to potential outbreaks of WNV
Contact Information

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