Mosquito Testing Program for Arbovirus Surveillance in Texas

Bethany Bolling, MS, PhD
Microbiologist, Arbovirus Laboratory

Texas Department of State Health Services, Austin, Texas
Overview

- Arbovirus surveillance
- Role of DSHS Arbovirus Laboratory
- Testing procedures
- Summary data
- Challenges and future directions
Mosquitoes are Hematophagous Insects

- Mosquito saliva contains:
  - Anticoagulants
  - Anesthetic
  - Vasodilators
  - Immunomodulators
  - Potential pathway for pathogen transmission
The World's Deadliest Animals
Number of people killed by animals per year

- Mosquito: 725,000
- Human: 475,000
- Snake: 50,000
- Other:
  - Dog (rabies): 25,000
  - Tsetse fly (sleeping sickness): 10,000
  - Assassin bug (Chagas disease): 10,000
  - Freshwater snail (schistosomiasis): 10,000

- Other animals:
  - Ascaris roundworm: 2,500
  - Tapeworm: 2,000
  - Crocodile: 1,000
  - Hippopotamus: 500
  - Elephant: 100
  - Lion: 100
  - Wolf: 10
  - Shark: 10

Source: Gatesnotes
Arboviruses
(Arthropod-borne Viruses)

- **Bunyaviridae**
  - La Crosse encephalitis virus
  - California encephalitis virus
  - Rift Valley fever virus
  - Crimean-Congo hemorrhagic fever virus

- **Flaviridae**
  - West Nile virus
  - St. Louis encephalitis virus
  - Dengue viruses
  - Japanese encephalitis virus
  - Yellow fever virus
  - Zika virus

- **Togaviridae**
  - Eastern equine encephalitis virus
  - Western equine encephalitis virus
  - Venezuelan equine encephalitis virus
  - Ross River virus
  - Chikungunya virus
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Importance of Mosquito Testing for Arbovirus Surveillance

- Emerging and reemerging diseases
- Lack of vaccines licensed for human use
- Lack of antiviral drugs or therapies
- Vector control is the primary disease prevention strategy
- Vector control
  - Costly
  - Toxicity issues
  - Difficulty treating large areas
TX DSHS Arbovirus Laboratory

• The objectives for our lab are:
  • to provide mosquito species identification (year-round)
  • to detect and track the circulation of arboviruses in mosquitoes prior to the development of human disease (May-November)
  • to test rabies-negative equines (also llamas and alpacas) for arboviruses (year-round)
Mosquito Collection and Submission

Gravid trap

Light trap

BG Sentinel trap

[Images of mosquito collection methods and submission process]
Mosquito Species Tested

**Culex species**
- Culex quinquefasciatus
- Culex tarsalis*
- Culex (Melanoconion)
- Culex salinarius
- Culex restuans
- Culex nigripalpus
- Culex stigmatasoma

**Aedes species**
- Aedes aegypti
- Aedes albopictus
- Aedes triseriatus
- Aedes trivittatus

**Culiseta species**
- Culiseta melanura

*Pooled separately in El Paso
**EEE activity: all species pooled and tested separately
Mosquitoes are pooled by species and placed in tubes.

Each tube is labeled with a pool# and contains 1 BB. Diluent is added for the homogenization process.
Mosquitoes are homogenized using a Mixer Mill

Mixer Mill blocks are loaded with tubes and placed into the machine where mosquito samples are homogenized.
Homogenized samples are centrifuged and supernatants are inoculated onto two vertebrate cell lines: Vero and BHK cells
Supernatants inoculated onto cell culture plates in a BSL3 Suite
Observation of Inoculated Cell Cultures

Cells checked daily for cytopathic effects (CPE) for 10 days post inoculation (dpi).
Cells are Scraped and Fixed to 5-well Slides
Immunofluorescent Antibody Test for Arbovirus Identification
Immunofluorescent Antibody Test for Arbovirus Identification

- WNV CPE generally shows up within 3-6 dpi
- Cells are scraped and IFA completed same day
- Positive arbovirus results are reported by phone and email
Current Testing Capabilities

- West Nile virus
- St. Louis encephalitis virus
- Eastern equine encephalitis virus
- Western equine encephalitis virus
- Venezuelan equine encephalitis virus
- Highlands J virus
- California group viruses (send to CDC for ID)
- Tensaw virus
- Chikungunya virus
- Pan-alphavirus, -bunyavirus, -flavivivirus
Arbovirus Positive Mosquito Pools, 2015 (as of 11-10-15)

- **SLEV Detected**
- **WNV Detected**
- **In-House Testing**
- **DSHS Testing**
Arbovirus Activity
2015
Report Date: November 10, 2015

*Arbovirus activity in a county is indicated on the map by any of the following: bird, mosquito pool, sentinel chicken, horse, human or presumptive viremic donor.
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<th>Year</th>
<th>Pools tested</th>
<th>Pools positive</th>
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<th>SLE</th>
<th>WEE</th>
<th>WN/ SLE</th>
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West Nile Virus Disease Cases and Deaths in Texas 2002-2015

Source: cdc.gov
## Mosquito Species Testing Positive 2004-2014

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<th>Mosquito Species Tested</th>
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Challenges and Future Directions

• Lacking geographically representative submissions
  • Increase county participation
• Limitations of cell culture surveillance
  • Increase capacity
  • Decrease turn-around time
Challenges and Future Directions

- Addition of molecular testing
  - Purchase equipment
  - Validate multiplex RT-PCR assays
- Detection of new or reemerging arboviruses
  - Continue broad cell culture surveillance
  - Target a small subset of vector species
Acknowledgements

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  • Mary D’Anton
  • Joanne Day
  • Joseph Hancock
  • Hima Rambhatla
  • Jeannie Xiang

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  • Dave Florin
  • Nicole Evert

• Submitting Agencies
  • City/County Health Dept.
  • Vector Control Programs
  • Universities
  • Military Installations