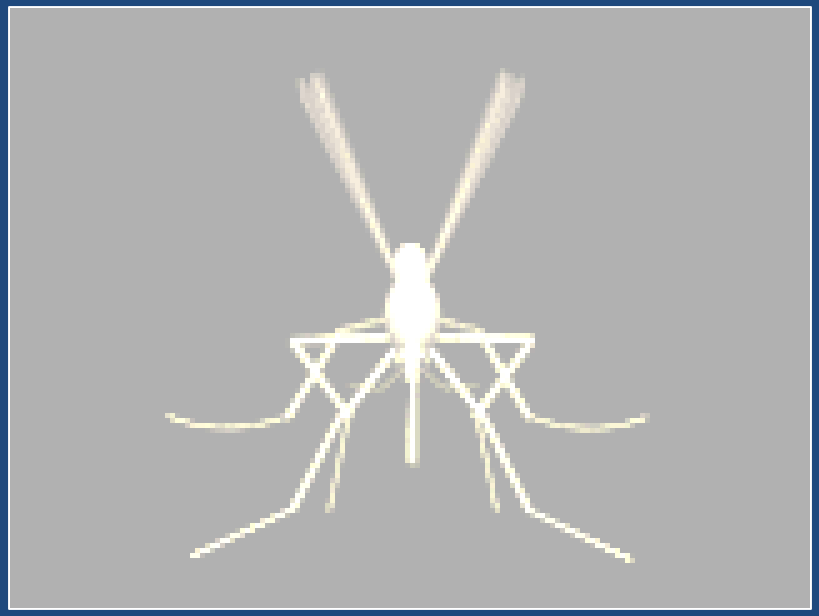
TEXAS
Department of State Health Services

Mosquito Testing Program for Arbovirus Surveillance in Texas



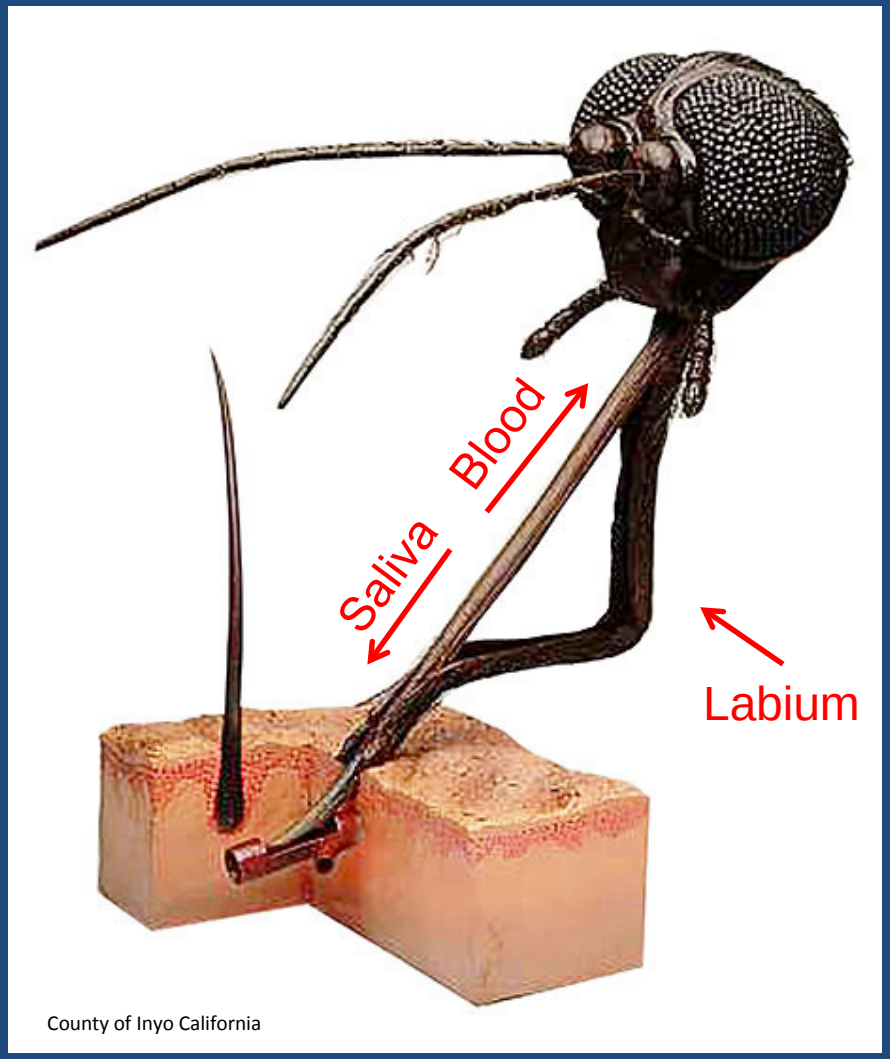
Bethany Bolling, MS, PhD
Microbiologist, Arbovirus Laboratory

Texas Department of State Health Services, Austin, Texas

Overview

- Arbovirus surveillance
- Role of DSHS Arbovirus Laboratory
- Testing procedures
- Summary data
- Challenges and future directions

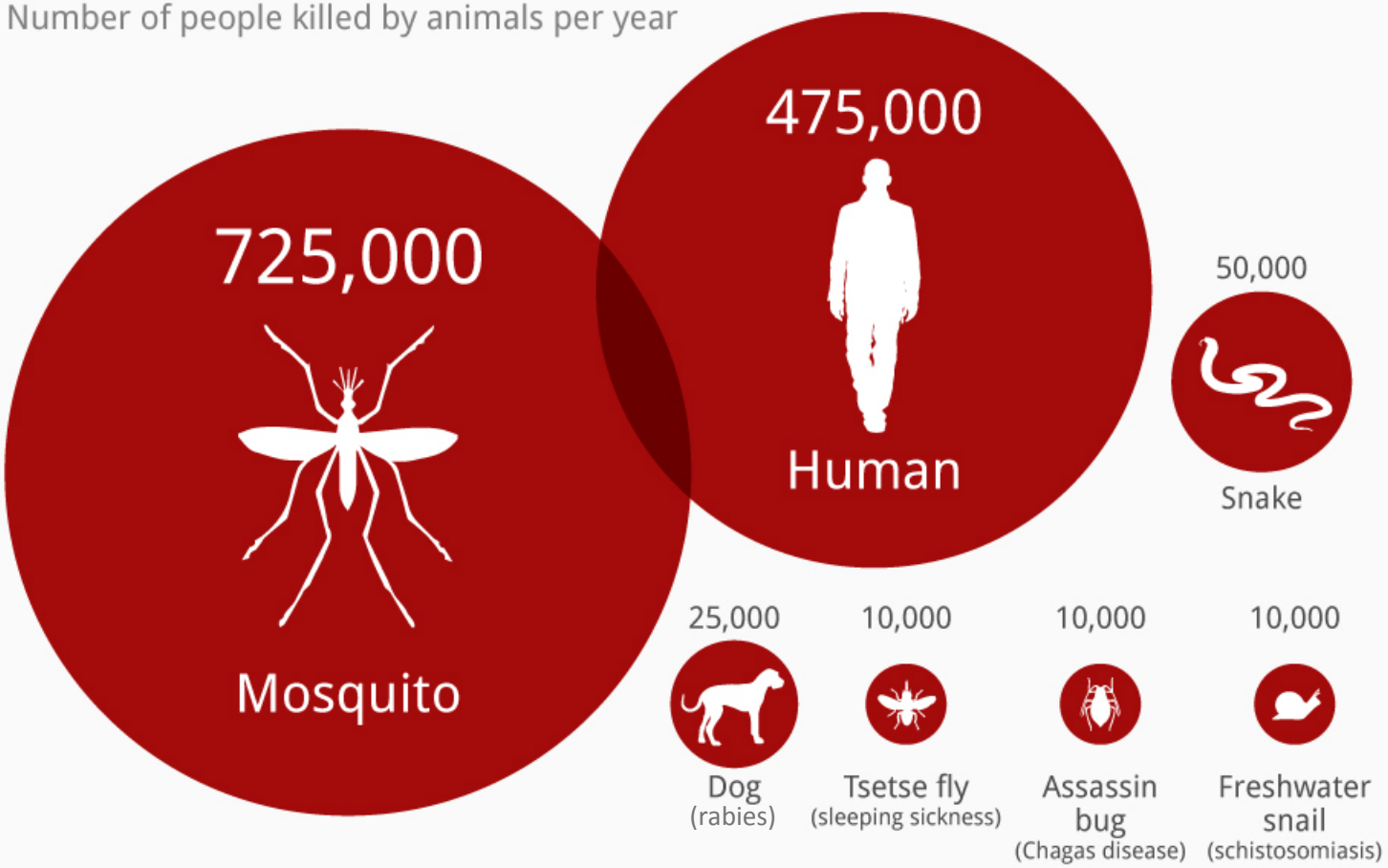
Mosquitoes are Hematophagous Insects



- 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



@StatistaCharts

Source: Gatesnotes

statista

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 Sentinal trap

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*

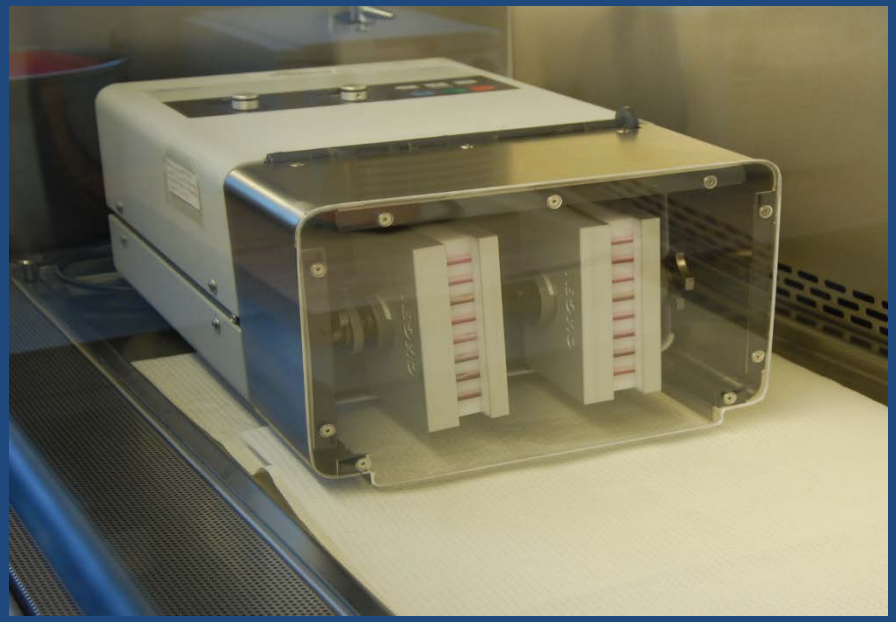
*Pooled separately in El Paso
**EEE activity: all species pooled and tested separately

Mosquitoes are pooled by species and placed in tubes

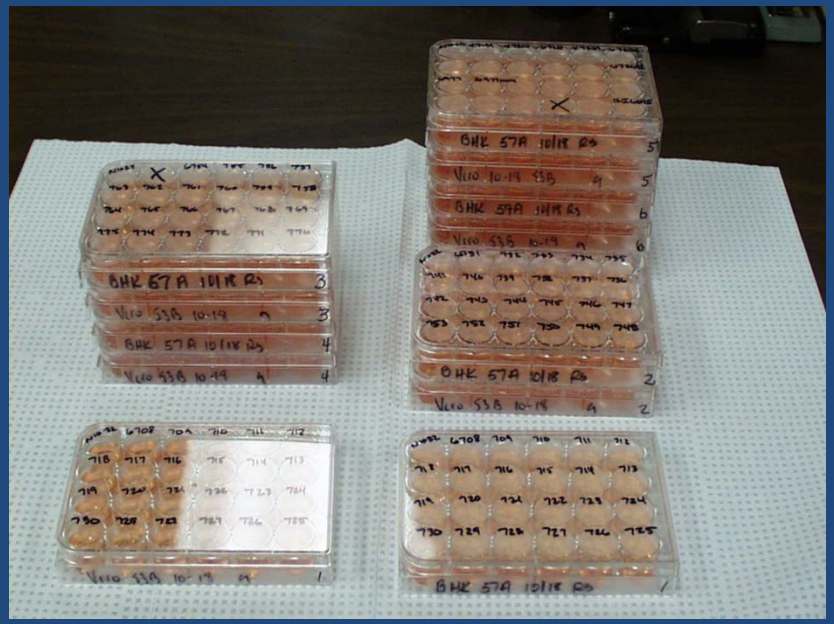
Each tube is labeled with a pool# and contains 1 BB. Diluent is added for the homogenization process.

Mosquitoes are homogenized using a Mixer Mill

Mixer Mill blocks are loaded with tubes and placed into the machine where mosquito samples are homogenized.



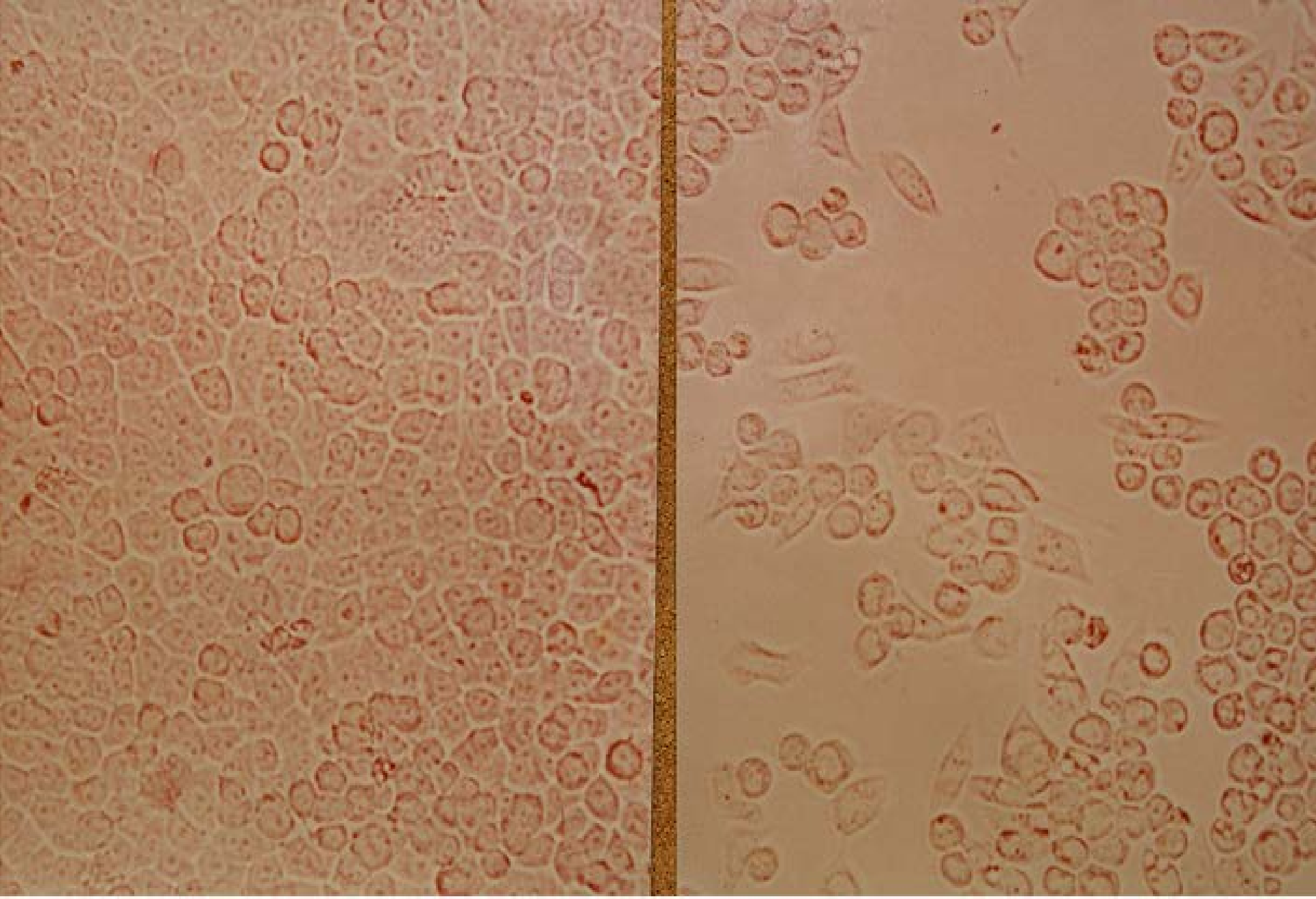
Homogenized samples are centrifuged and supernatants are inoculated onto two vertebrate cell lines: Vero and BHK cells



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).

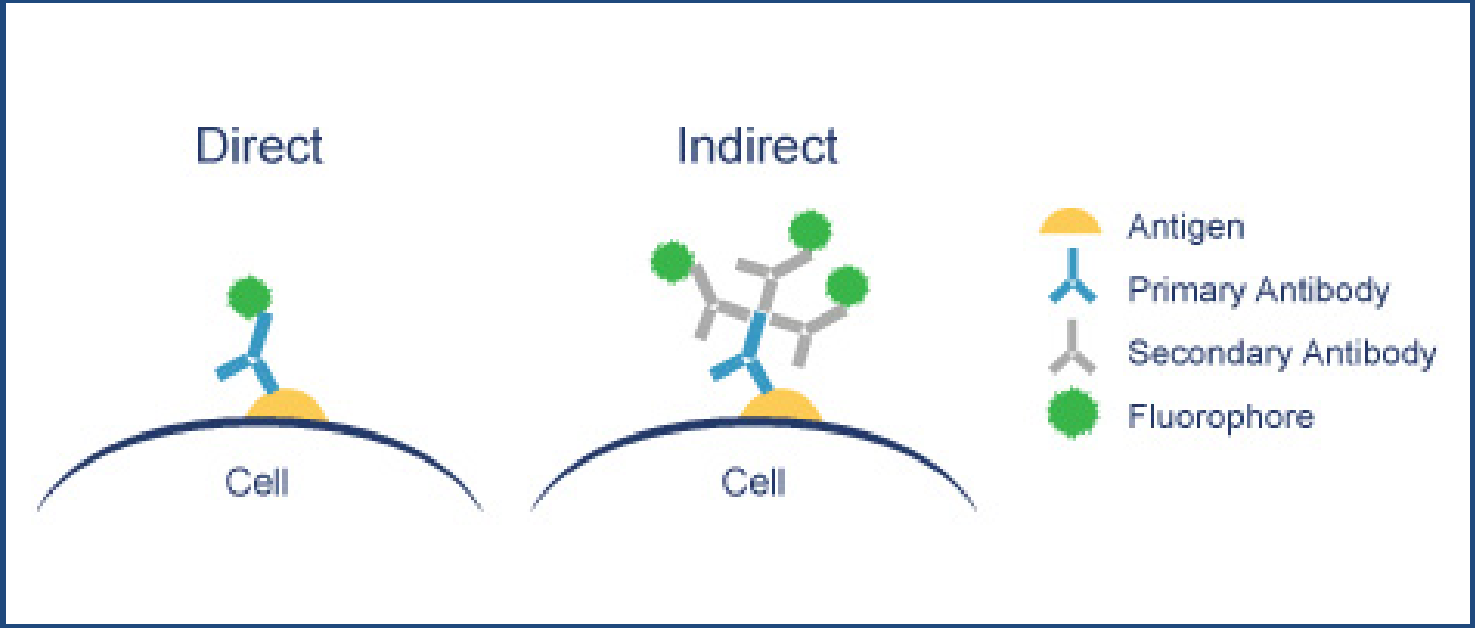


Photographs of normal tissue culture-Left side

Photographs of infected tissue-Right side

Cells are Scraped and Fixed to 5-well Slides

Immunofluorescent Antibody Test for Arbovirus Identification



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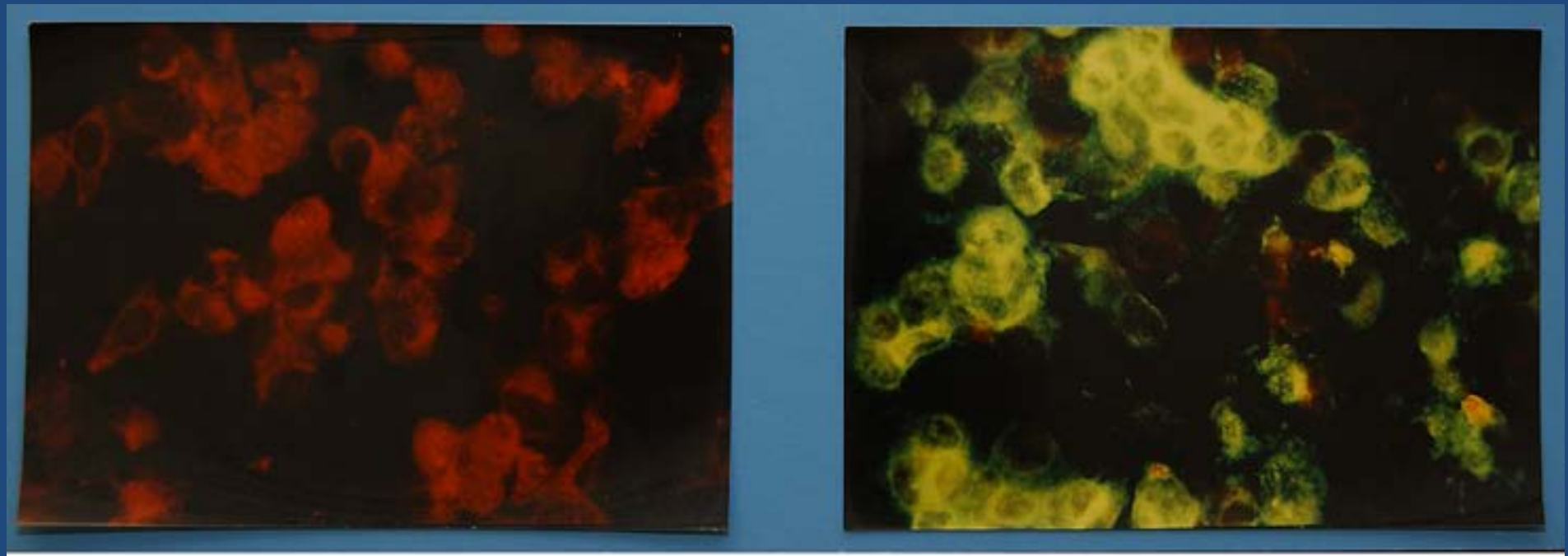

Photographs of negative fluorescent antibody test used to identify viruses-Left side

Photographs of positive fluorescent antibody test used to identify viruses-Right side

- 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, -flavivirus

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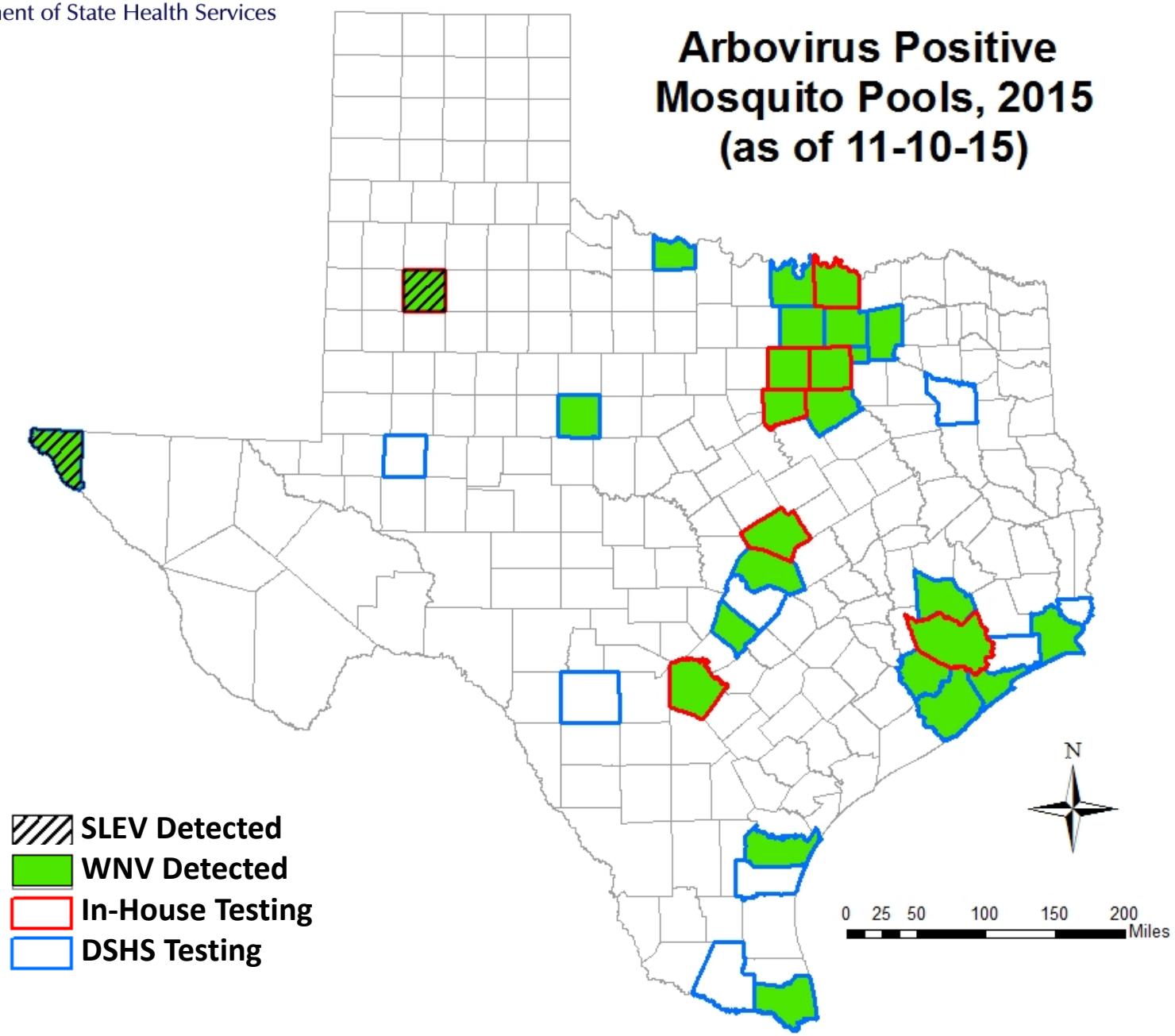
Arbovirus Positive Mosquito Pools, 2015 (as of 11-10-15)



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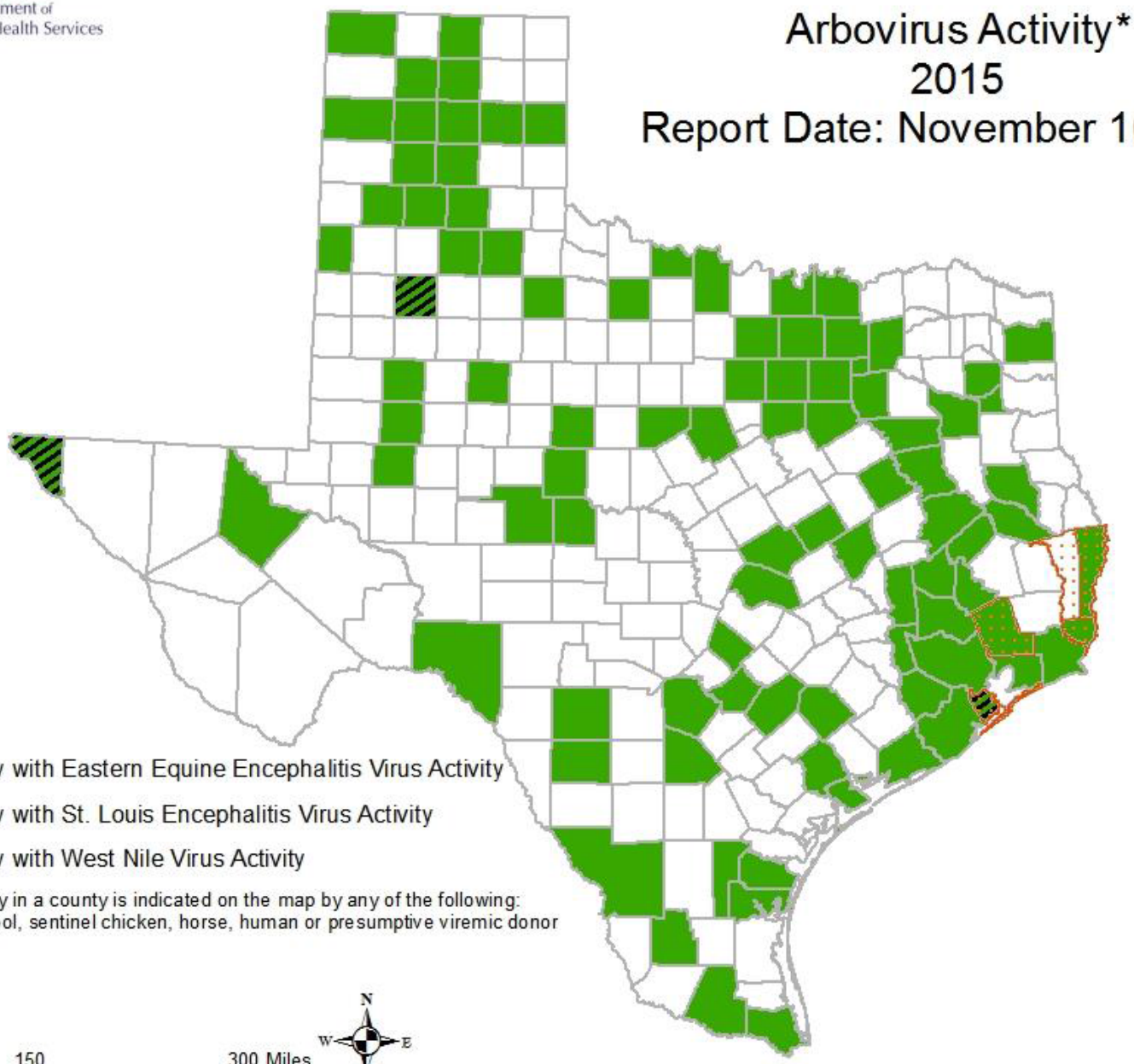
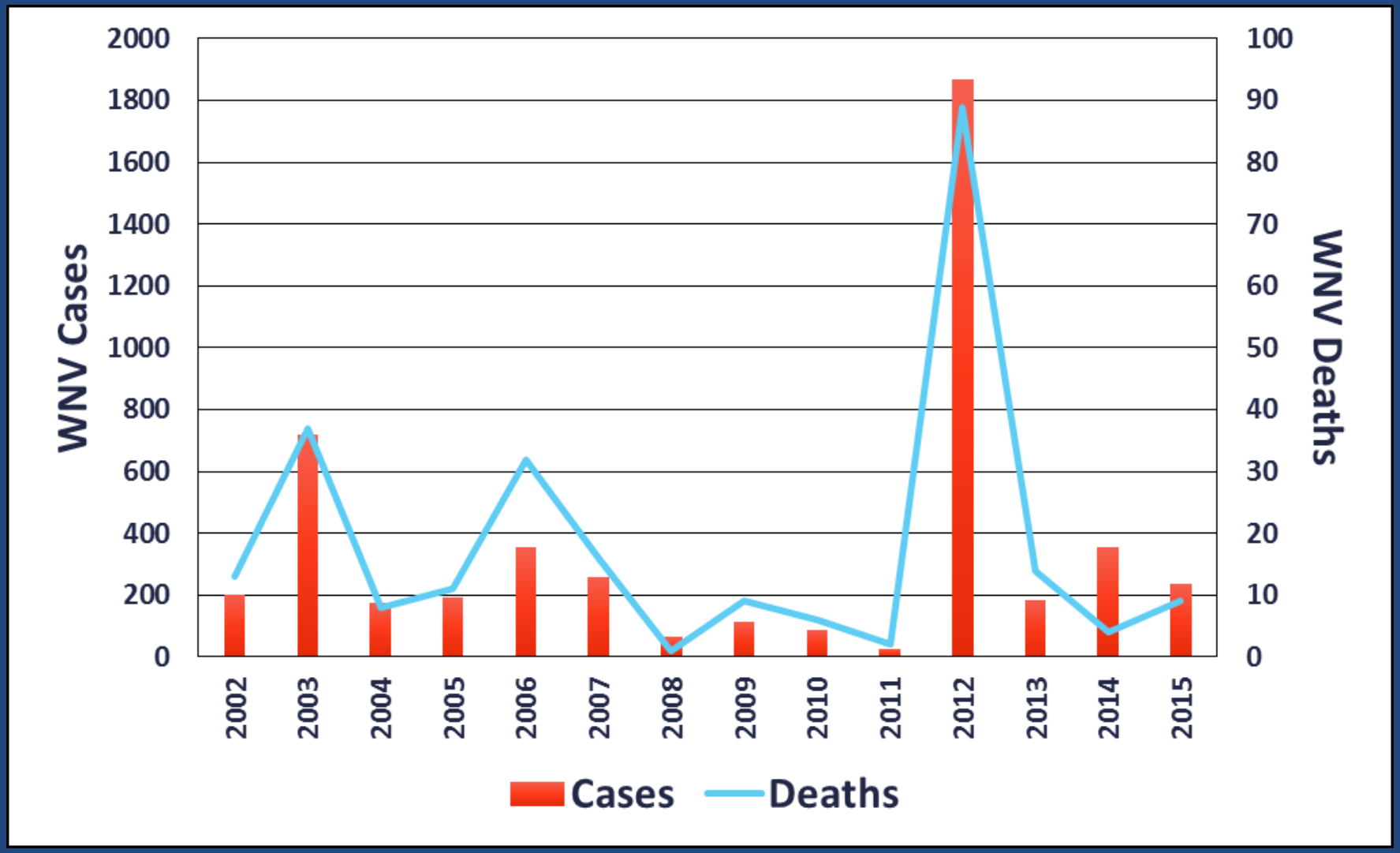
Arbovirus Activity*
2015
Report Date: November 10, 2015



Positive Pools 2002-2014

Year	Pools tested	Pools positive	WN	SLE	WEE	WN/ SLE	Other
2002	7,814	466	189	229	10	34	4
2003	10,814	839	836	1			2
2004	10,951	170	164		2		4
2005	9,779	328	309		10		5
2006	9,567	438	432				6
2007	11,074	226	223				3
2008	9,580	93	92				1
2009	9,850	161	144	6			11
2010	8,997	22	16	6			
2011	8,473	52	52				
2012	11,863	803	798				5
2013	11,998	75	73	2			
2014	12,158	326	320	6			
2015	14,500	248	232	3			13

West Nile Virus Disease Cases and Deaths in Texas 2002-2015



Source: cdc.gov

Mosquito Species Testing Positive 2004-2014

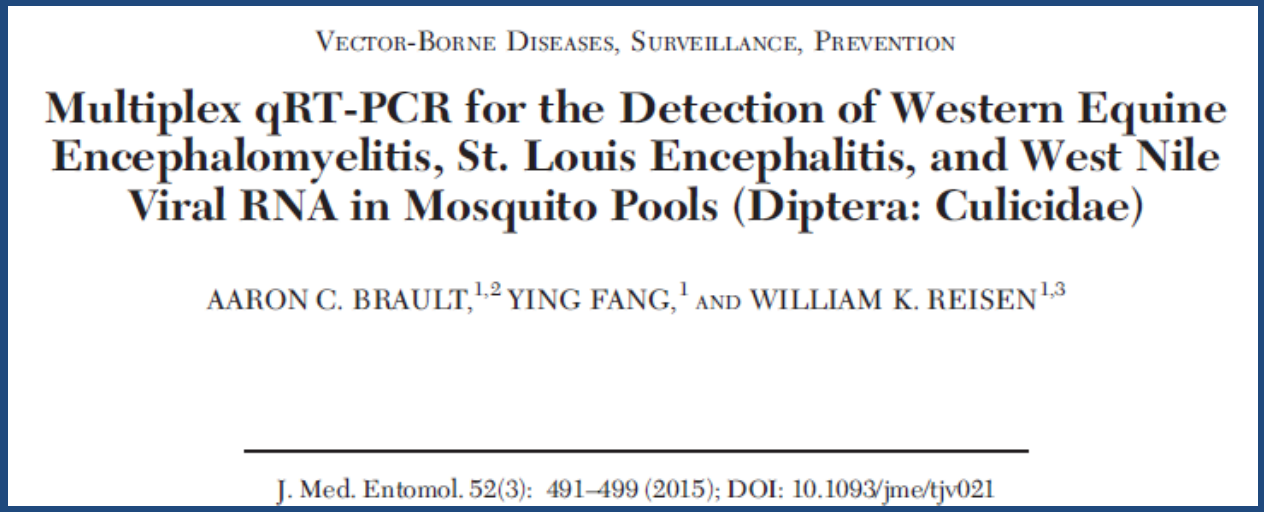
Mosquito Species Tested	LACV	SLEV	WEEV	WNV	Total
Aedes aegypti				4	**4**
Aedes albopictus	1			28	**26**
Aedes taeniorhynchus				1	**1**
Aedes triseriatus	1			1	**2**
Aedes trivittatus					**0**
Culex (Melanoconion)			1	41	**42**
Culex nigripalpus				7	**7**
Culex quinquefasciatus		18	1	2444	**2463**
Culex restuans				6	**6**
Culex salinarius				7	**7**
Culex species				2	**2**
Culex stigmatosoma					**0**
Culex tarsalis		2	5	57	**64**
Total	**2**	**20**	**7**	**2598**	**2627**

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



VECTOR-BORNE DISEASES, SURVEILLANCE, PREVENTION

Multiplex qRT-PCR for the Detection of Western Equine Encephalomyelitis, St. Louis Encephalitis, and West Nile Viral RNA in Mosquito Pools (Diptera: Culicidae)

AARON C. BRAULT,[1,2] YING FANG,[1] AND WILLIAM K. REISEN[1,3]

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  - Vector Control Programs
  - Universities
  - Military Installations