La Crosse Encephalitis in Eastern Tennessee

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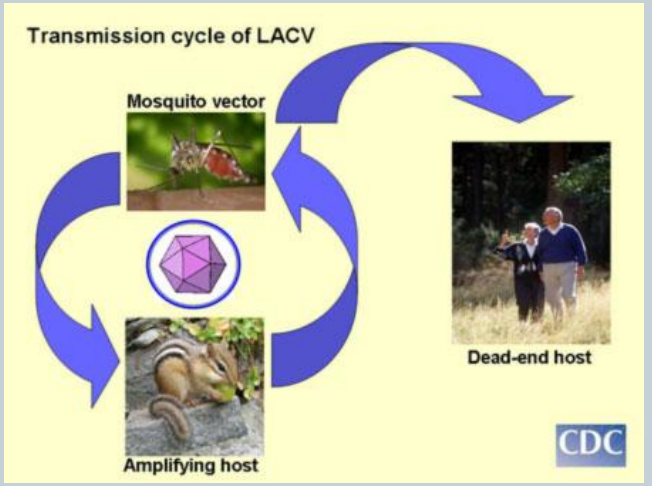
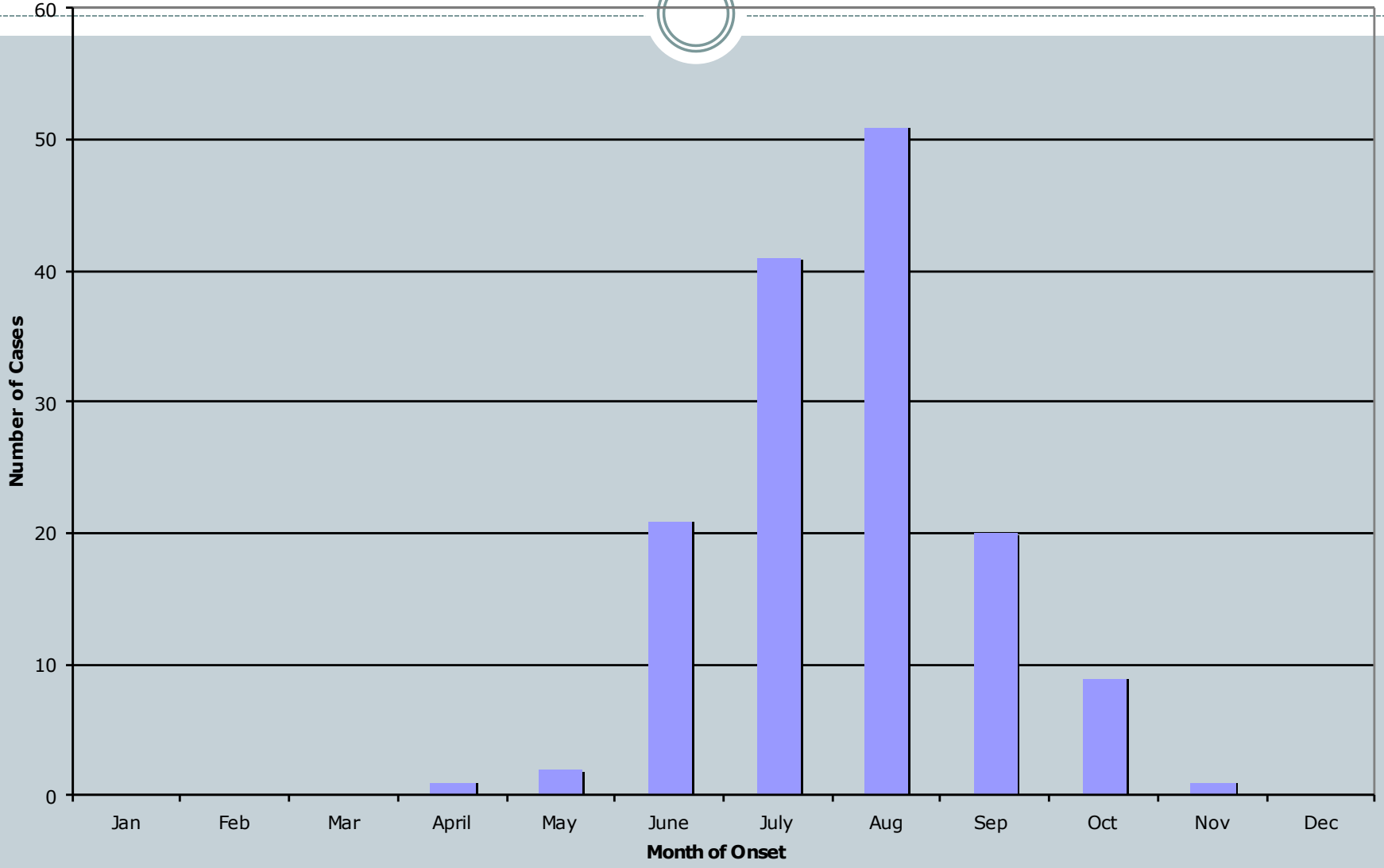
Clinical Presentation and Epidemiology

- Fever, headache, nausea, vomiting, fatigue, lethargy.
- Meningitis, encephalitis
- Neurological sequelae: recurrent seizures, hemiparesis, cognitive and neurobehavioral abnormalities
- Case fatality less than 1%
- Supportive therapy (including seizure control)

- Most cases in children under 16
- Average 80-100 cases reported per year
- Most cases July - September
- Boys mostly affected

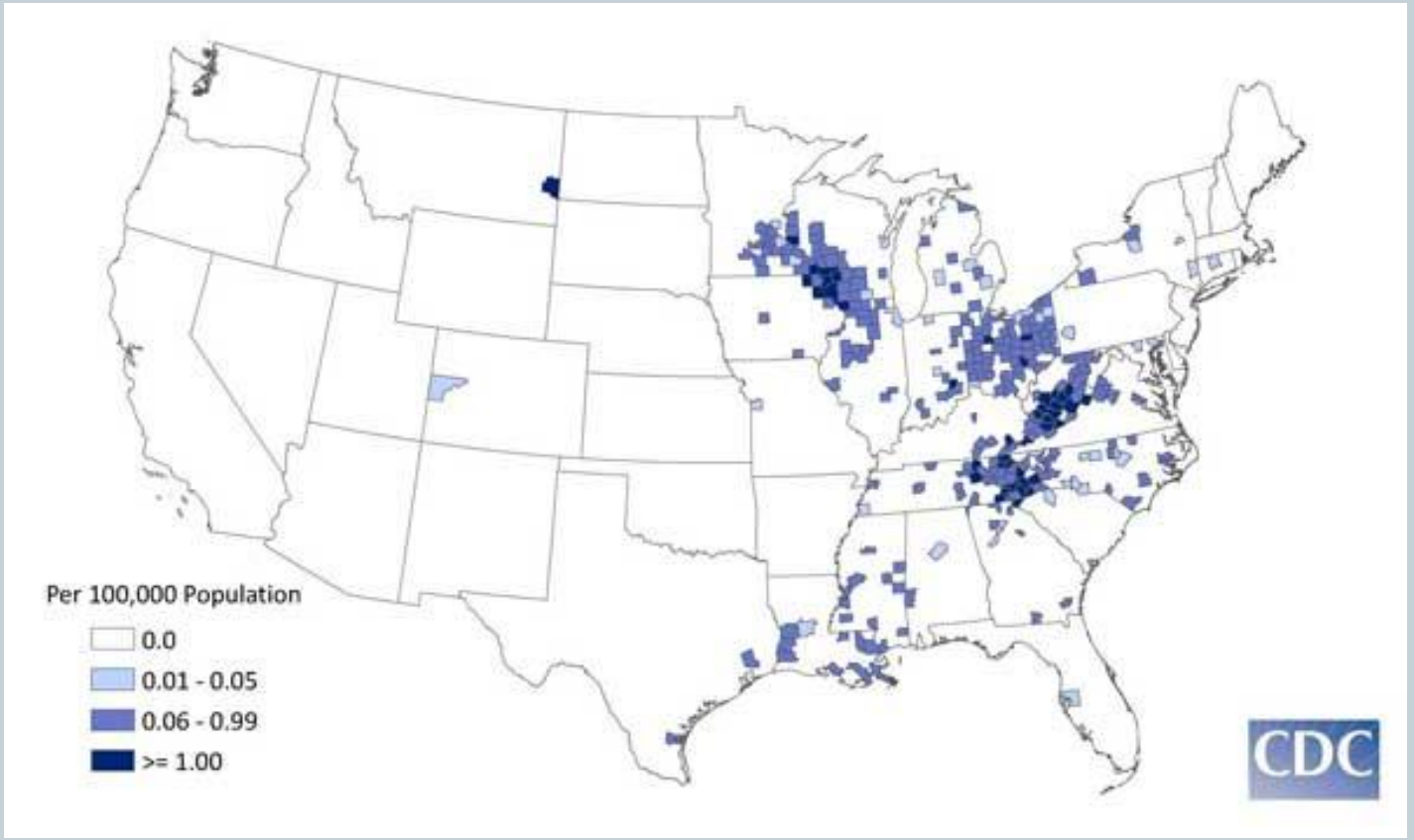


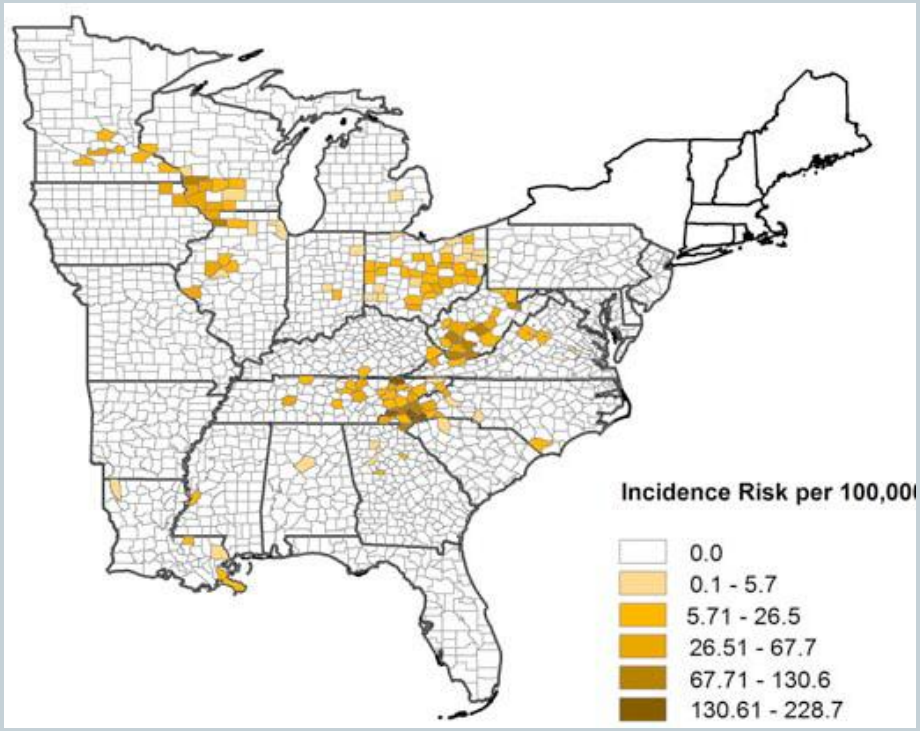
Distribution of La Crosse Encephalitis Cases, by Month of Onset, Tennessee, 1998-2010



California Serogroup Virus Neuroinvasive Disease Cases, 1964-2010

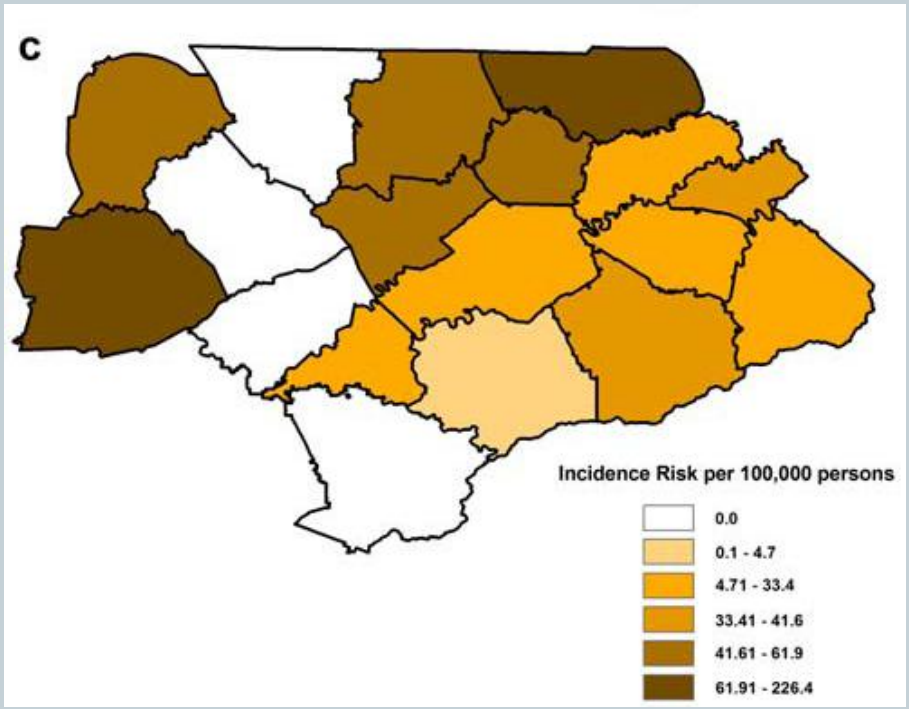
California Serogroup Virus Neuroinvasive Disease Average Annual Incidence by County, 1996-2010





Unsmoothed risk at county level for children 15 and under, 2003-2007

Haddow, AD, Odoi A (2009) The Incidence Risk, Clustering, and Clinical Presentation of La Crosse Virus Infections in the Eastern United States, 2003-2007. PLoS ONE 4(7): e6145.

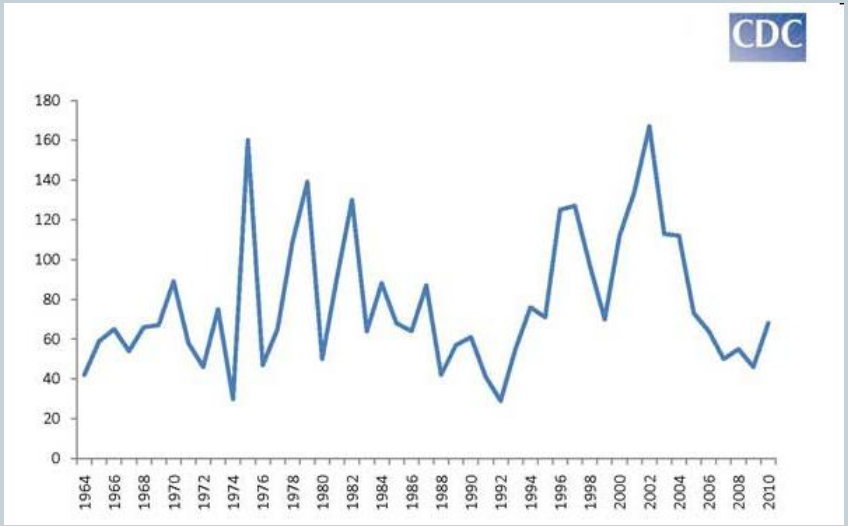


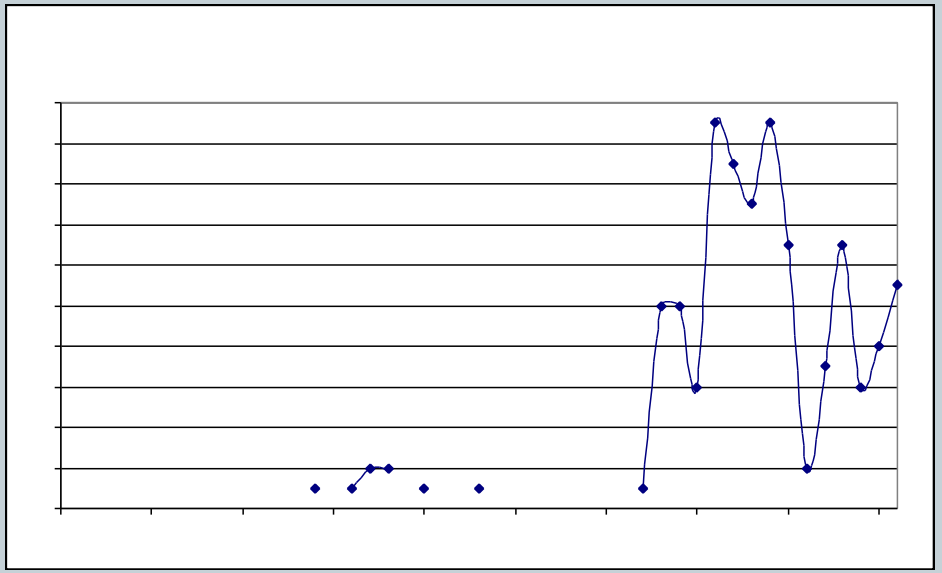
Distribution of unsmoothed risk of La Crosse virus infections at the county level for eastern Tennessee of population 15 years and younger, 1997-2006

Haddow AD, Jones CJ, Odoi A (2009) Assessing Risk in Focal Arboviral Infections: Are We Missing the Big or Little Picture? PLoS ONE 4(9): e6954.

Emergence in Tennessee

- From 1964-1996, only 9 cases in TN
- 1997 cluster of 10 cases in eastern TN
- Cases increased about the same time in WV and NC
- Over 160 cases reported in TN





Vectors

- *Ochlerotatus triseriatus*
  - Treehole mosquito
  - Primary vector
- *Aedes albopictus*
  - Asian tiger mosquito
  - New introduction
- *Ochlerotatus japonicus*
  - Asian bush mosquito
  - Newer introduction

Hypothesis

- These mosquito species may differ in their relative contribution to the maintenance and transmission of LACV to humans
  - Differences in abundance at case sites
  - Differences in infection rates at case sites
  - Differences in blood meal composition at case sites
- Some traps are better than others for capturing specific species

Methods

Selection of Study Sites

- Case reports were obtained for all LACV cases from 2004-2009
- Cases were mapped and clusters identified
- Calls were made to households in the order of most recent and in our preferred counties (Knox and Claiborne)
- Six households were enrolled representing five cases

Egg Collections and Rearing

- Five standard oviposition cups were set at each site
- The eggs are removed each week and returned to the lab
- Eggs are reared to adults in a temperature controlled environmental chamber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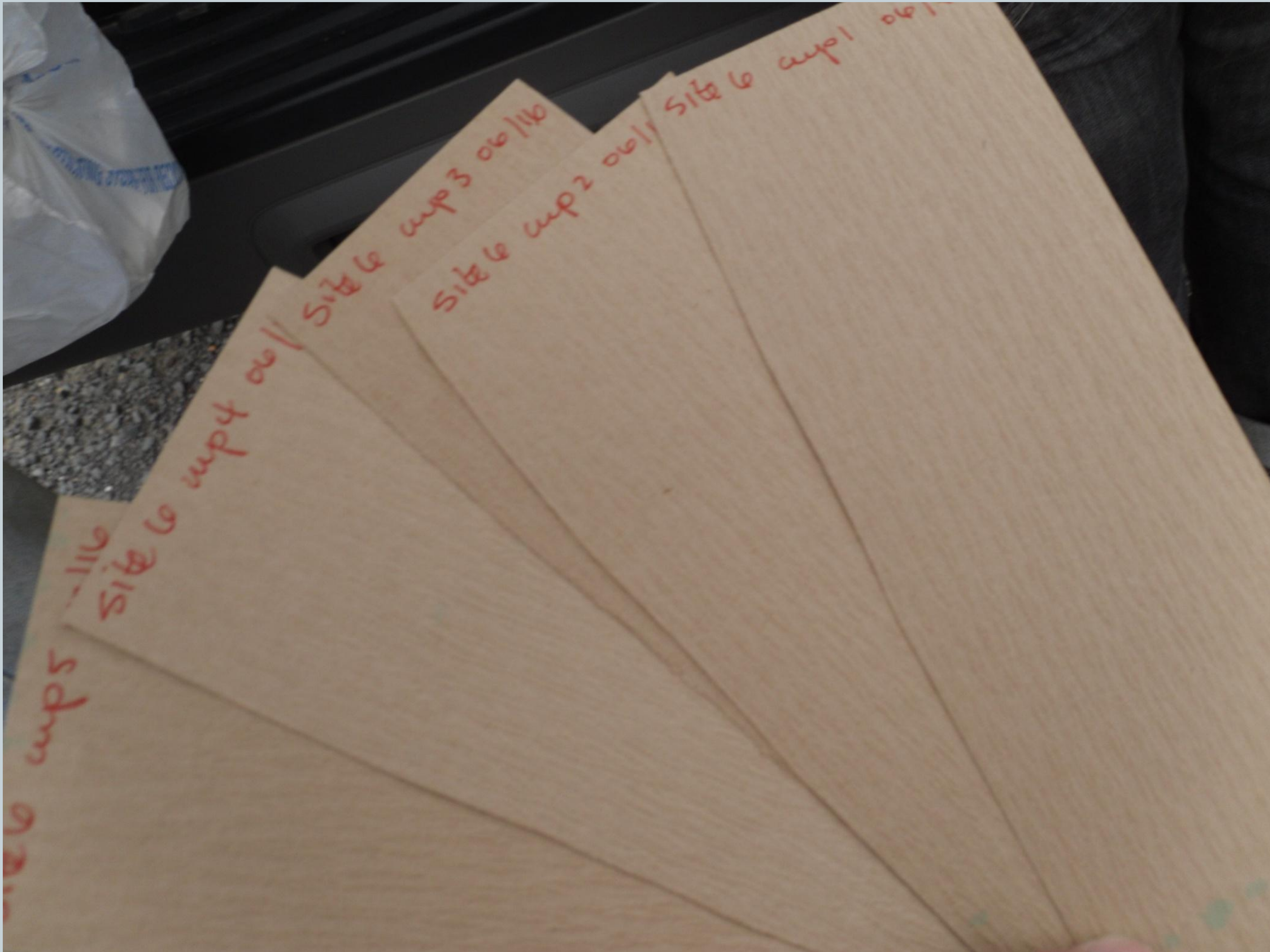
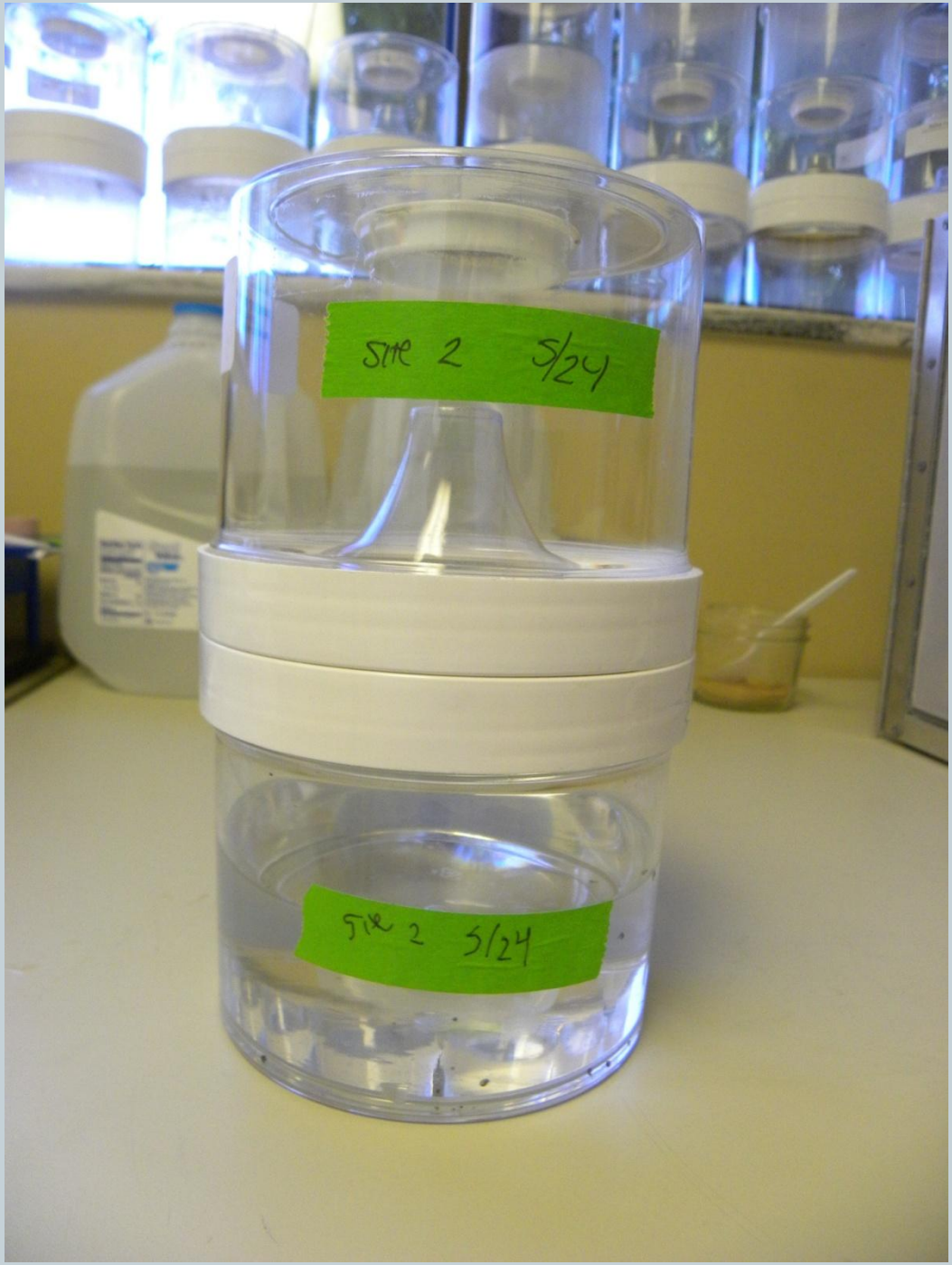

Site 6 cup1 06|
Site 6 cup2 06|
Site 6 cup3 06|16
Site 6 cup4 06|
Site 6 cup5


Site 1 5/24


Site 2 5/24
Site 2 5/24

Larval Collections

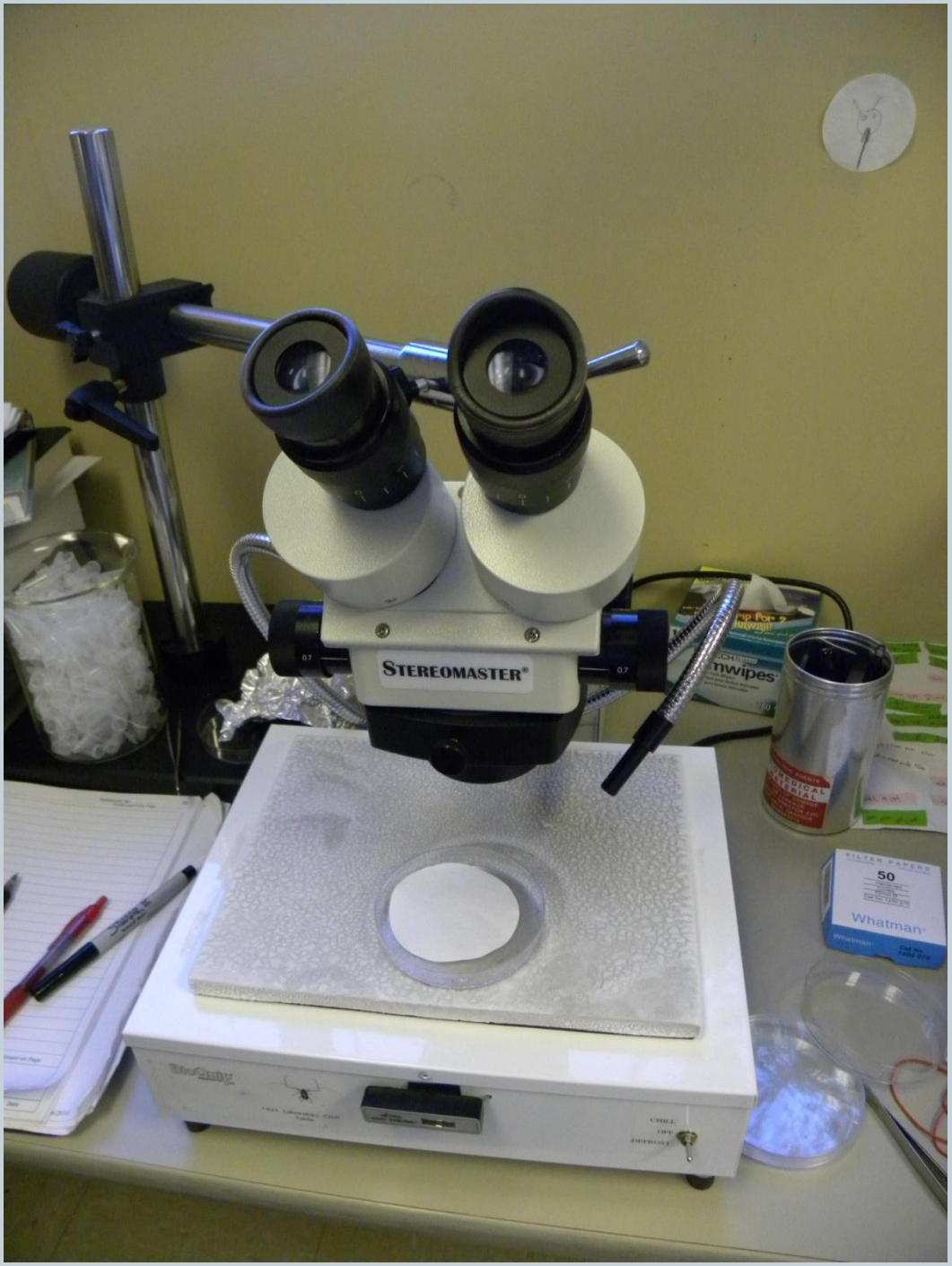
- All standing water on the field sites is examined every week for larvae/ pupae
- If present, a sample is collected and returned to the lab for rearing

Adult Collections

- Adults are collected in 2 ways: the BG Sentinel Trap and by aspirating
- The BG trap is set every week and run for approximately 24 hours
- Each site is aspirated, using either the Prokopack or the CDC Backpack Aspirator, for 20-40 minutes 1 to 4 days a week
- Adults are transported back to the lab on dry ice

Identification and Storage

- Adults are identified, sorted and numbered using a compound microscope and a chill table
- They are separated into pools of ≤ 23 mosquitoes by site, date, species and sex
- Stored in a -80 C chamber


STEREOMASTER
Whatman
50


LAC mosquitoes working box

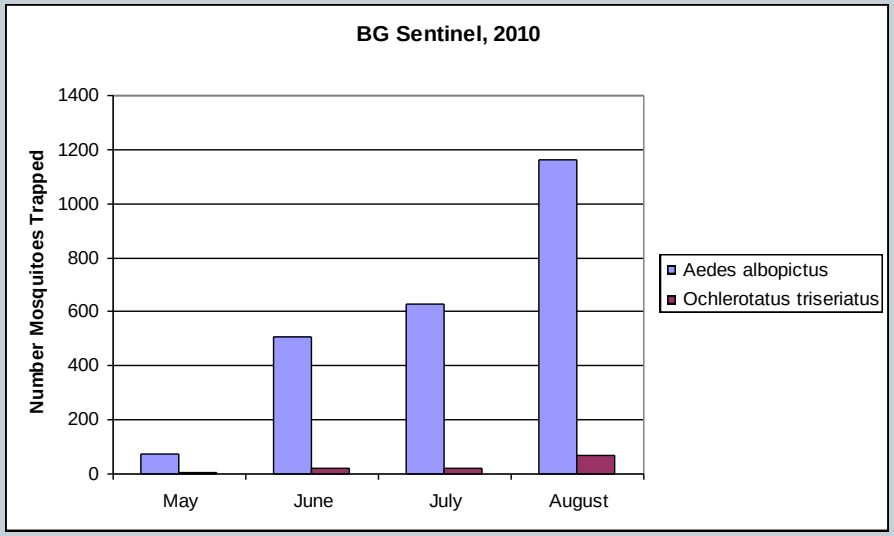
RNA Extraction and Viral Testing

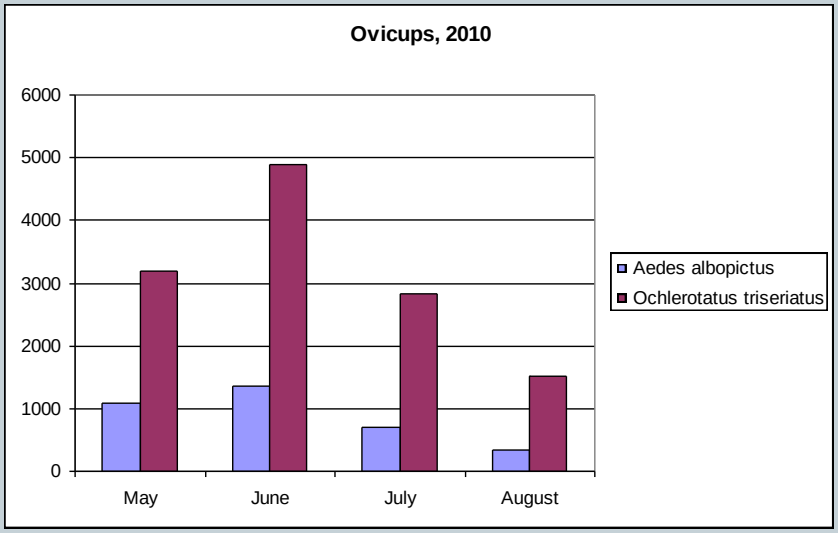
- Mosquito pools are homogenized in cell culture media
- RNA is extracted using QIAamp Mini RNA Extraction Kit or Biogents Robot
- RT- PCR is run using the protocol from Kuno et al. 1996
- The primers screen for 24 different viruses in the Bunyamwera/ California groups, including JC
- Some samples have also been tested using cell culture

Species per trap

Trap	Species	# mosquitoes
Prokopack	*Oc. triseriatus*	36
	Ae. albopictus	276
	Oc. japonicus	0
BG trap	*Oc. triseriatus*	321
	Ae. albopictus	2374
	Oc. japonicus	94
Ovitrap	*Oc. triseriatus*	12400
	Ae. albopictus	3485
	Oc. japonicus	1306
Larval collections	*Oc. triseriatus*	320
	Ae. albopictus	183
	Oc. japonicus	208

Mosquitoes Collected





Infected *Ochlerotatus triseriatus* (ovicups)

Date	Site	Gender	# Pools
6/9/2010	1	M	6
6/9/2010	1	F	4
6/22/10	5	M	2
6/22/10	5	F	2
7/12/10	6	M	2
7/19/10	1	M	1
7/19/10	2	F	3
7/19/10	3	M	3
7/19/10	5	M	2
8/9/10	5	M	2
8/9/10	6	F	2
8/19/10	3	F	8
8/19/10	5	M	2

Other LACV Infected Mosquitoes

Species	Gender	Site	#/pool	Date
Ochlerotatus japonicus	F	6	20	5/19/2010
Aedes albopictus	F	1	3	5/24/2010
Aedes albopictus	M	3	13	6/1/2010
Aedes albopictus	F	5	18	7/7/2010
Aedes albopictus	M	6	9	7/12/2010
Aedes albopictus	M	1	5	7/19/2010
Aedes albopictus	M	3	8	7/19/2010
Aedes albopictus	F	1	7	7/27/2010
Aedes albopictus	F	5	18	8/9/2010
Aedes albopictus	M	5	17	8/9/2010

Bloodmeal

- What are they eating?
  - Samples:15 *Aedes albopictus*, 2 *Ochlerotatus triseriatus*, 1 *Ochlerotatus japonicus*
  - Tested for mammalian and avian bloodmeals

Hypothesis

- These mosquito species may differ in their relative contribution to the maintenance and transmission of LACV to humans
  - Differences in abundance at case sites
    - Trap dependent, *Oc. triseriatus* > *Ae. abopictus* > *Oc. japonicus*
  - Differences in infection rates at case sites
    - 11 LACV ID events for *Oc. triseriatus* and 8 for *Ae. albopictus*
    - 1 LACV ID event for *Oc. japonicus*
  - Differences in blood meal composition at case sites
    - Testing pending
- Some traps are better than others for capturing specific species
    - *Oc. triseriatus* = Ovicups
    - *Ae. albopictus* = Ovicups/BG
    - *Oc. japonicus* = Ovicups

Conclusions

- 1st ID of LACV in *Oc. triseriatus* in TN
- 1st ID of LACV in *Oc. japanicus* in U.S.
- 2nd ID of LACV in *Ae. albopictus* in TN (3rd in U.S.)
- For surveillance
  - BG may be useful for *Ae. albopictus*
  - Ovicups for *Oc. triseriatus* and *Oc. japonicus*
- Prokopack good for bloodmeal collections in *Ae. albopictus*
- Larval collections representative of populations
- PCR more sensitive than cell culture assays

Acknowledgements

- TDH
  - Katie Westby
  - Melissa Heim
  - Erin Westby
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  - Charissa Fritzen
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- The families in east TN

Questions?