

INVESTIGATING THE DIVERSITY OF ARBOVIRUSES AND MOSQUITOES AT THE NASHVILLE ZOO AT GRASSMERE

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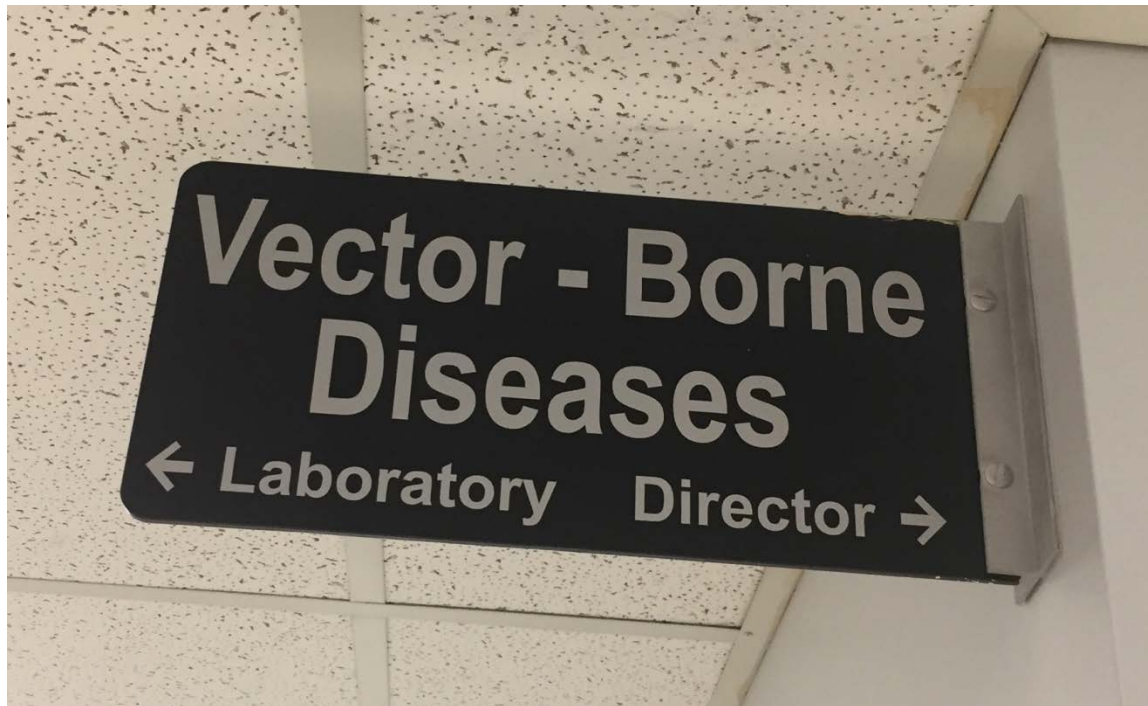
It all started with a Bontebok...



September 3, 2017, a male bontebok deceased, while presenting signs of encephalopathy.

Testing the Bontebok

- Received and tested bontebok brain tissue
 - qRT-PCR showed positivity for West Nile Virus (WNV) in cerebellum and spinal cord.
 - Conventional RT-PCR confirmed WNV



What's the Big Deal?

- To our knowledge, this animal species has never shown neuro-invasive signs of WNV infection.
- The Nashville Zoo is a recreational place for the community
 - The general public interact with the animals



Taking the Next Steps

Objective:

- Identify mosquitoes around the Nashville Zoo
- Identify the prevalence of WNV among captured mosquitoes

Mosquito traps were set throughout the zoo

CO₂ CDC Light Traps



Gravid Traps



BG Sentinel traps



Oviposition Cups





- Food**
- 1 Snake Bites/BBQ Depot
 - 2 Zoofari Cafe
 - 3 Outpost Snack Bar

- Rides (tickets required)**
- 1 Wilderness Express
 - 2 Soaring Eagle
 - 3 Wild Animal Carousel

- Private Rental Areas**
- 1 Botswana Overlook
 - 2 Jungle Terrace



Summary of Adult Mosquito Collections

Mosquito	Count
<i>Culex pipiens</i>	2881
<i>Aedes albopictus</i>	122
<i>Aedes trivittatus</i>	929
<i>Aedes vexans</i>	61
<i>Psorophora</i> sp.	5
Total	3998

Unique Findings

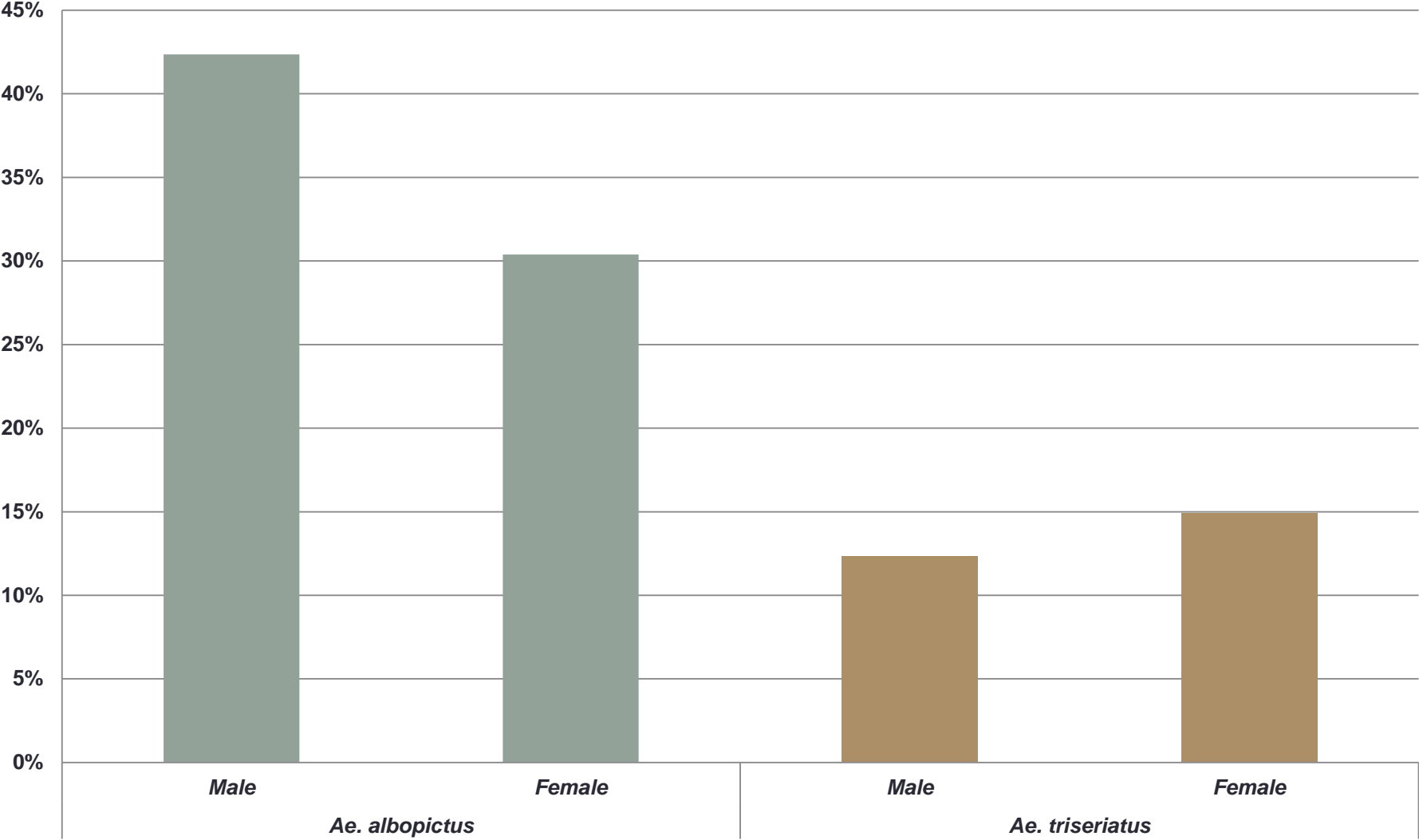
- Lots of *Ae. trivittatus* and *Ae. vexans*
- Not a lot *Ae. albopictus*

Summary of Egg Collections

- Set up oviposition cups at 4 locations between 9/25 to 10/3
- Mosquitoes eggs were reared to adults at the TDH Vector-borne Diseases Program's insectory



Mosquitoes hatched from the Nashville Zoo at Grassmere, 2017



Arbovirus Testing

- Multiplex qRT-PCR
 - WNV
 - St. Louis encephalitis Virus (SLEV)
 - Flanders Virus (FLAV)
- La Crosse Virus qRT-PCR
 - Only tested *Ae. triseriatus*
- Conventional RT-PCR
 - WNV confirmation



Nashville Zoo at Grassmere Mosquito Trapping Sites, Nashville, TN, September 19 - October 13, 2017



Legend

- FLAV
- SLEV
- WNV
- Mosquito Trap Sites
- ▭ Bontebok Exhibit
- ▭ Nashville Zoo At Grassmere Property Boundary

0 0.05 0.1 0.2 Miles

This map was generated using ArcGIS 10.2.2 (Esri, Redlands, CA) using imagery provided by the Tennessee Department of Transportation. November 13, 2017



Summary of Arbovirus Results

- No *Aedes* species showed positivity
- 6/13 sites showed arbovirus positivity among mosquitoes
 - 2/6 of the sites were by the bontebok site
 - 4/6 of the sites were where avian species were stored
- The most WNV positivity was found near lorikeets
- The most SLEV wasn't found near any zoo animals
 - However, the most SLEV found near zoo animals was by the cranes and bontebok.
- The most FLAV was found near the cranes and bontebok
- There was no La Crosse Virus

Summary of Arbovirus Results

Pathogen	Positive Pools (%)	MLE ^α	CI (95%)
WNV	18.06	10.82	(7.35, 15.45)
FLAV	9.03	5.08	(2.91, 8.30)
SLEV	8.39	4.68	(2.62, 7.79)

^α Maximum Likelihood Estimate (MLE) is based on a scale per 1,000, using a biased-corrected point estimate with a corrected score 95% confidence interval (CI).

Unique Findings

- There's SLEV!
- There's a lot of WNV!

Comparison of WNV Results from September 19 - October 13, 2017

Region	% Positive	MIR*	CI (95%)	MLE*	CI (95%)
Tennessee	17.75	4.29	(3.67, 4.91)	4.74	(4.09, 5.47)
Shelby Co.	16.73	3.54	(2.91, 4.16)	3.87	(3.87, 4.60)
Memphis	13.10	2.72	(2.03, 3.40)	2.91	(2.24, 3.72)
Nashville w/ Zoo	20.25	8.03	(5.77, 10.29)	9.23	(6.89, 12.15)
Nashville w/o Zoo	28.57	6.90	(3.89, 9.91)	8.23	(5.18, 12.58)
Zoo	18.06	9.72	(6.14, 13.30)	10.82	(7.35, 15.45)

* MIR and MLE are based on estimated number of mosquitoes per 1,000 mosquitoes.

Potential Reasons



Zoo animals reservoir the virus?

Wildlife reservoir in the park?



Zoo habitat conducive for mosquitoes?

Taking Action

- Results, thus far, have been disseminated to Zoo leadership and pest control management.
- Continued collaboration with veterinary staff to continue testing and studying the zoo animals
 - Potential necessity of vaccine use.
- Suggested mosquito control methods to pest control staff.
- Future communication of repellent use to staff and visitors.
 - Increased mosquito nuisance

Future Research

Explore different capture methods for *Ae. triseriatus*

Identify other possible affected mammals

- Antibody neutralization tests for other unvaccinated zoo animals for WNV

Exploring SLEV

- Genetic analysis to identify the strain

What are the mosquitoes feeding on?

- Genetic analysis of fed mosquito biome

Are there other arboviruses circulating through the Zoos mosquito population?

- Trivittatus Virus, etc.

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Thank You