

Project Title: Enhanced Surveillance of Arboviruses in Florida
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The goal of enhancing surveillance of arboviruses in Florida was addressed with the following two specific aims:

Aim 1: Assess arboviral activity in three counties of Florida.

In completing Aim 1, we developed an alternative approach to testing mosquito pools for arboviruses, utilizing a *superpooling* strategy. This method decreases the costs associated with arbovirus testing of mosquito pools by a factor of approximately 10. This reduced cost is especially important when dealing with large sample numbers. Briefly, mosquitoes are sorted and pooled using standard protocols. When mosquito samples are processed for extraction of viral RNA and subsequent virus testing via quantitative RT-PCR (qRT-PCR), we draw and then combine 0.025 mL from each of 10 pools, instead of drawing 0.250 mL from each pool. Then, each 0.250 mL sample is added to 0.250 mL of extraction buffer prior to processing. This allows us to test 10 times as many samples at once. When a virus-positive superpool sample is found, each of the 10 pools that comprise this sample is tested individually in order to find the specific positive sample. Utilizing this method, we successfully identified positive laboratory samples and mosquito pools collected from Florida in 2005 (Table 1).

The current study was carried out in conjunction with sentinel chicken serosurveillance conducted by an NIH-funded study "Modeling and empirical studies of arboviruses in Florida". Using superpooling, we carried out arboviral testing of mosquitoes collected from chicken-baited traps in the 2006 field season. For the current study, we tested mosquitoes associated with chickens that did not produce viral antibodies when exposed to mosquitoes. Mosquitoes associated with chickens that did produce viral antibodies were planned to be tested using the NIH-funded study "Modeling and empirical studies of arboviruses in Florida". However, no sentinel chickens were found positive for viral antibodies in 2006, possibly due to a state-wide decrease in arboviral activity. Mosquitoes were sorted into 6,476 mosquito pools containing = 50 mosquitoes / pool. The predominant mosquito species was *Culex nigripalpus*. We have tested 5,440 pools, of which 11 tested positive for an arbovirus using the superpooling method (Table 2). These samples were found in all three counties throughout the trapping period (June-October) and need to be confirmed.

Aim 2: Develop a multiplex qRT-PCR assay for simultaneous detection of multiple arboviruses.

Based on previous studies utilizing multiplex assays for the detection of flaviviruses, we customized a multiplex qRT-PCR assay to simultaneously detect West Nile virus (WNV) and St. Louis encephalitis virus (SLEV). This provided a cost-saving benefit by initially screening field samples for both viruses. The two probes used in this reaction emit fluorescence at different wavelengths, allowing mosquito pools potentially containing both SLEV and WNV to be processed simultaneously, while recording fluorescence emission at both frequencies. The primers and probes used were specific to SLEV or WNV and no cross-hybridization was detected in preliminary laboratory studies. However, our dual probe system could not detect SLEV when high titers of WNV were present. Accordingly, all WNV-positive samples were later assayed for SLEV using a single probe test for final verification.

These data support previous studies that indicate sentinel animals can be exposed to virus positive mosquitoes and not produce viral antibodies. We have also initiated development of two methods for testing mosquito pool samples that will greatly reduce both the time and cost associated with testing mosquitoes for SLEV and WNV. These methods should enhance future surveillance efforts for arboviruses in Florida.

A manuscript addressing the superpooling method by Daniel Chisenhall, Christopher Vitek, Stephanie Richards, and Christopher Mores, was submitted for review to Journal of the American Mosquito Control Association in June 2007. We anticipate an additional manuscript detailing the results from testing the field collected mosquitoes. Results from this research will be presented at the annual meeting of the Florida Mosquito Control Association in 2007 by Christopher Vitek.

Table 1. Samples used to compare qRT-PCR detection of WNV using standard- versus super-pooling methods.

Standard sample ¹	Result	Superpool Sample ¹	Result
<u>Laboratory Derived</u>			
0.250 mL negative pool	-	0.025 mL from each of 10 negative pools	-
0.250 mL <i>low</i> pool	+	0.025 mL <i>low</i> pool + 0.225 mL negative pool	+
0.250 mL <i>medium</i> pool	+	0.025 mL <i>medium</i> pool + 0.225 mL negative pool	+
0.250 mL <i>high</i> pool	+	0.025 mL <i>high</i> pool + 0.225 mL negative pool	+
<u>Field Derived (2005 samples)</u>			
Positive pools*	+	0.025 mL positive pool + 0.225 mL negative pool	+
<u>Negative controls</u>			
0.50 mL buffer	-	0.50 mL buffer	-
<u>Positive controls</u>			
0.250 virus stock	+	0.250 virus stock	+

¹0.25 mL buffer was added to all samples for a total volume of 0.5 mL

*Tested previously under other protocols.

+Samples found WNV-positive by qRT-PCR.

Table 2. Positive superpools from field collected mosquitoes in 2006.

Superpool	Site	Date	Mosquito Species	Virus [†]
1	Duval County/Indian River County*	6/27/06	<i>Cx. quinquefasciatus</i> , <i>Cx.</i> <i>erraticus</i> , <i>Cx. nigripalpus</i> , <i>Cq.</i> <i>perturbans</i> , <i>Cx. salinarius</i>	SLEV
2	Manatee County	7/13/06	<i>Cx. nigripalpus</i>	WNV
3	Manatee County	7/13/06	<i>Cx. nigripalpus</i>	WNV
4	Manatee County	7/13/06	<i>Cx. nigripalpus</i>	WNV
5	Manatee County	7/13/06	<i>Cx. nigripalpus</i>	WNV
6	Manatee County	8/17/06	<i>Cx. nigripalpus</i>	WNV
7	Manatee County	8/17/06	<i>Cx. nigripalpus</i> , <i>Cx. erraticus</i> , <i>Oc. infirmatus</i>	WNV
8	Manatee County	9/8/06	<i>Cx. nigripalpus</i> , <i>Cx. erraticus</i> , <i>Oc. infirmatus</i> , <i>Ma. dyari</i>	WNV
9	Indian River County	9/26/06	<i>Cx. nigripalpus</i>	WNV
10	Duval County	9/26/06	<i>Cx. nigripalpus</i> , <i>Cx. salinarius</i> , <i>Cx. erraticus</i> , <i>Oc. infirmatus</i>	WNV
11	Indian River County	10/3/06	<i>Cx. nigripalpus</i>	WNV

*This superpool consisted of pools from both Indian River County and Duval County.

[†]These results from initial screening of superpools must undergo confirmatory testing.